IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

~~17721~~

MGIPC—S4—III-1-93—22-8-45—5,000.

JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 69

JULY 1-DECEMBER 15, 1944

316715
IARI

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1945

For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.
Price 5 cents

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

P. N. ANNAND, Chairman
Chief, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration

JOHN W. ROBERTS
Principal Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration

BENJAMIN SCHWARTZ
Principal Zoologist, Chief, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

V. R. GARDNER
Director, Michigan Agricultural Experiment Station

C. B. HUTCHISON
Director, California Agricultural Experiment Station

W. H. MARTIN
Director, New Jersey Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL

Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to V. R. Gardner, Director, Michigan Agricultural Experiment Station, East Lansing, Mich., who is chairman of the Station subcommittee.

Published with the approval of the Director of the Budget. Issued on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal: Domestic, $2.25 a year (2 volumes)
Foreign, $3.75 a year (2 volumes)
Single numbers: Domestic, 10 cents
Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at varying prices depending on their size. When purchased in quantity of 100 or more, a reduction of 25 percent in the cost will be made. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

Vol. 69 Washington 25, D. C. July 1–December 15, 1944

CONTENTS

Page

Smut Resistance in an *Allium* Species Hybrid. J. C. Walker, H. A. Jones, and A. E. Clarke 1

Effects of Soil Treatments on the Growth of the Highbush Blueberry. Illus. T. A. Merrill 9

Potato Varieties in Relation to Blackening after Cooking. Illus. G. H. Rieman, W. E. Tottingham, and John S. McFarlane 21

Chemical Composition of Some American Wild Feedstuffs. Thomas R. King and Harold E. McClure 33

Comparison of Shoot Apex and Leaf Development and Structure in Diploid and Tetraploid Maize. Illus. L. F. Randolph, Ernst C. Abbe, and John Einset 47

Damping-off in Broadleaf Nurseries of the Great Plains Region. Illus. Ernest Wright 77

Anatomical and Cytological Studies on Beet Mosaic. Illus. Katherine Esau 95

Reaction of *Lactuca* Species to the Aster Yellows Virus under Field Conditions. Ross C. Thompson 119

Differential Effects of Temperature on the Development of the Beet Leafhopper. Illus. F. H. Harries 127

Relation of Cultivation to Depletion of Root Reserves in European Bindweed at Different Soil Horizons. Illus. A. L. Bakke, W. G. Gaessler, L. M. Pultz, and S. C. Salmon 137

Additional Strains of the Sugar-Beet Curly Top Virus. Illus. N. J. Giddings 149

Biology of *Allotropa burrelli*, a Gregarious Parasite of *Pseudococcus comstocki*. Illus. D. W. Clancy 159

Variation and Physiologic Specialization in the Common Scab Fungus (*Actinomyces scabies*). Illus. L. A. Schaal 169

Acquired Immunity from Curly Top in Tobacco and Tomato. Illus. James M. Wallace 187

Moisture Retention by Some Irrigated Soils as Related to Soil-Moisture Tension. Illus. L. A. Richards and L. R. Weaver 215

Deficiency, Toxicity, and Accumulation of Boron in Plants. Illus. Frank M. Eaton 237

Factors Influencing Embryonation and Survival of Eggs of the Stomach Worm, *Haemonchus contortus*. Doys A. Shorb 279

Studies on the Use of the Point-Quadrat Method of Botanical Analysis of Mixed Pasture Vegetation. William B. Drew 289

Regression of Insect Density on Distance from Center of Dispersion as Shown by a Study of the Smaller European Elm Bark Beetle. Illus. F. M. Wadley and D. O. Wolfenbarger 299

The Ring Rot Bacterium in Relation to Tomato and Eggplant. Illus. R. H. Larson 309

Analysis of Variation in *Panicum virgatum*. Illus. Etlar L. Nielsen 327

Some Effects of the Waxy and Sugary Genes on Endosperm Development in Maize. Illus. R. H. Andrew, R. A. Brink, and N. P. Neal 335

The Fermentation of Cigar-Leaf Tobacco as Influenced by the Addition of Yeast. M. F. Gribbins, D. E. Haley and J. J. Reid 373

Value of Soybean Meal Prepared from Damaged (Bin-Burned) Soybeans as a Feed for Growing Swine. J. L. Krider, B. W. Fairbanks and W. E. Carroll 383

Page

Bionomics of the Lymnaeid Snail, *Stagnicola bulimoides techella*, the Intermediate Host of the Liver Fluke in Southern Texas. Illus. O. WILFORD OLSEN 389
A Study of Dormancy and Germination of Seeds of *Cercis canadensis*. Illus. MICHEL AFANASIEV 405
Development and Survival under Outdoor Conditions of Eggs and Larvae of the Common Ruminant Stomach Worm, *Haemonchus contortus*. A. G. DINABURG 421
Meiotic Studies of Crosses between *Fragaria ovalis* and × *F. ananassa*. LEROY POWERS 435
Some Host-Parasite Relations in the Black Root Rot of Apple Trees. Illus. J. S. COOLEY 449
Effectiveness of Selection on Progeny Performance as a Supplement to Earlier Culling in Livestock. Illus. G. E. DICKERSON and L. N. HAZEL 459

ERRATA AND AUTHORS' EMENDATIONS

Page 108, line 4, "mosaic and diseased" should be "mosaic-diseased."
Page 333, fig. 2, C, near top, large black irregularly shaped symbol between symbol for decaploid (●) and symbol for tetraploid (✳) should be eliminated.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., JULY 1, 1944 No. 1

SMUT RESISTANCE IN AN ALLIUM SPECIES HYBRID[1]

By J. C. WALKER,[2] *agent*, H. A. JONES, *principal olericulturist*, and A. E. CLARKE, *associate cytologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Smut (*Urocystis cepulae* Frost) of onion (*Allium cepa* L.) is widely distributed and is of considerable economic importance in the northern onion-growing sections of the United States. Only in the seedling stage is the host susceptible to attack, since all infection takes place through the cotyledon, which was found by Anderson (*1*)[3] to be susceptible until about the time the first leaf emerges. Walker and Jones (*11*) found that the infection takes place at soil temperatures between 10° and 27° C. and that the disease develops better at soil and air temperatures of 15° and 20° than at temperatures above 20°. Walker and Wellman (*12*) later showed that the optimum temperature for germination of chlamydospores and hyphal fragments of the pathogen was between 13° and 20°, while the mycelium grew best at 18°.

Onion smut in commercial fields is controlled by applying formaldehyde in the row at the time of seeding. Nevertheless, the development of resistant varieties would be of value, since their use would eliminate considerable labor and expense. Anderson (*2*) tested 54 varieties of cultivated onions and since none showed any marked resistance to the disease, it appears probable that all commercial varieties of *Allium cepa* are susceptible to invasion by the smut organism. However, in a test of 39 other species of *Allium*, 8 appeared immune. Seed obtained from Germany under the name "Winterbeck Zwiebel" proved highly resistant, since only 2 or 3 percent of the seedlings developed smut in the cotyledons and none was seen in any later stage. Anderson's description of this variety suggests that it belongs to *A. fistulosum* L. Felix (*6*) also found that the Nebuka type of *A. fistulosum* was highly resistant to smut. Evans (*4*) traced the host-parasite relation during the growth of the cotyledon of *A. cepa* and found the development of an increasing intracellular protoplasmic resistance as the cotyledon approached maturity. In a later study with *A. fistulosum* (*5*) he found the same defense mechanism more highly developed in the lower half of the cotyledon. This accounted for the low incidence of infection below the point of emergence of the first leaf. When infection occurred above this point systemic invasion of the true leaves was precluded.

Allium cepa and *A. fistulosum* cross fairly readily, and interspecific hybrids have been obtained by Emsweller and Jones (*3*), Levan (*8, 9*), and Maeda (*10*). Unfortunately these hybrids have been highly self-sterile, but recently Jones and Clarke (7) obtained a fertile amphidiploid from a cross between these two species. *A. fistulosum* is then a source of smut resistance that may be incorporated into commercial varieties of onions. The behavior of these hybrids and their backcross generations to *A. cepa* in relation to smut resistance is presented in this paper.

---

[1] Received for publication March 30, 1943. Cooperative investigation of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, and the Department of Plant Pathology, Wisconsin Agricultural Experiment Station.

[2] Also professor, Department of Plant Pathology, Wisconsin Agricultural Experiment Station.

[3] Italic numbers in parentheses refer to Literature Cited, p. 7.

MATERIALS AND METHODS

The crossing was done in the greenhouses of the Bureau of Plant Industry Station, Beltsville, Md., and the testing for resistance was handled in the greenhouses of the Wisconsin Agricultural Experiment Station, Madison, Wis. In these tests Nebuka was the only type of *Allium fistulosum* used. Commercial varieties of *A. cepa* were obtained from various seed firms. The amphidiploid was the one reported by Jones and Clarke (7). It was obtained from a cross between the variety Australian Brown of *A. cepa* and Nebuka.

For the resistance test, thoroughly infested soil was obtained from a field in southeastern Wisconsin. The infestation was enhanced by the incorporation of spores from infected plants collected in the same area. Infested soil 3 inches deep was placed in greenhouse flats 20 by 14 by 3½ inches. Seed was sown in furrows three-fourths inch deep and 4 inches apart and covered; the usual greenhouse watering followed. The air temperature averaged about 22° C. and the soil temperature slightly below 20°.

Seedlings were grown until the cotyledon was fully developed and the first leaf about half grown. They were then removed carefully, all soil was washed off, and an examination was made for the presence and location of lesions. The diseased plants were placed in one of three classes, depending upon the location of the lesion, as follows:

(1) Lesion on cotyledon only above the point of emergence of the first leaf (crotch). Plants so affected practically always continue healthy.

(2) Lesion on cotyledon at or very near the crotch. Such plants may or may not escape systemic infection.

(3) Lesions on cotyledon below the crotch. Such plants usually become infected more or less systemically.

EXPERIMENTAL RESULTS

In 1936–37, 1,844 plants of *Allium fistulosum* were tested for smut resistance, and only 111, or 6.0 percent, developed lesions. All the lesions were above the crotch where they did little injury, and the attacked seedlings were able to survive. Plants of 3 varieties of *A. cepa* grown as controls were affected as follows: Of 88 plants of Ebenezer, 86.4 percent were diseased; of 108 plants of Sweet Spanish, 73.2 percent; and of 92 plants of Early Grano, 68.5 percent. The difference in amount of infection is actually more marked than these figures indicate, because, as a rule, infected seedlings of *A. cepa* develop a majority of the lesions below the crotch. In later tests plants were classified according to the position of the smut lesion in relation to the crotch of the cotyledon and first leaf.

No tests were made in 1937–38, but in 1938–39, as shown in table 1, Nebuka again proved highly resistant. Only 0.9 percent of the plants of this variety developed smut lesions below the crotch, in contrast with 74.9 percent of seven varieties of *Allium cepa*. The hybrids between *A. cepa* and Nebuka also showed considerable resistance, as only 13.7 percent of these plants developed lesions below the crotch. The percentage of infected plants in the hybrids was higher than in Nebuka but much lower than in the commercial varieties of *A. cepa*, indicating that the factor or factors for smut resistance are incompletely dominant.

Since the F_1 diploid plants were self-sterile it was not possible to obtain an F_2 population. The hybrids when used as the male parent in backcrosses to *Allium cepa* gave some seed. Unfortunately, these backcross populations were too small to justify drawing definite conclusions regarding the mode of inheritance of smut resistance, but it does seem that the percentage of plants which developed lesions below the crotch was much higher than in the F_1 populations. Seedlings that survived this test were retained for further breeding studies.

Similar results were obtained in 1939–40. These data are summarized in table 2. The percentages of seedlings which developed smut lesions below the crotch were 0.6, 61.2, and 8.7 for *Allium fistulosum*, *A. cepa*, and their F_1 hybrids, respectively. The first and second

TABLE 1.—*Results of testing commercial onion varieties, Nebuka, F_1 hybrid progenies, and first and second backcrosses to onion for resistance to smut in the greenhouse at Madison, Wis., 1938–39*

Variety or pedigree	Plants tested	Plants not smutted	Plants smutted: Total	Plants smutted: With lesion in indicated position: At or above crotch	Plants smutted: With lesion in indicated position: Below crotch	Tested plants smutted below crotch
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
Allium cepa (commercial varieties):						
Australian Brown	73	11	62	15	47	64.4
Crystal Wax	310	13	297	56	241	77.8
Southport Red Globe	38	11	27	13	14	36.8
Southport Yellow Globe	29	0	29	0	29	100.0
White Portugal	82	8	74	15	59	72.0
Yellow Bermuda	17	0	17	2	15	88.2
Yellow Globe Danvers	24	0	24	0	24	100.0
Total	573	43	530	101	429	74.9
A. fistulosum (Nebuka)	215	196	19	17	2	0.9
F_1 (*A. cepa* × *A. fistulosum*):						
Australian Brown × Nebuka	501	136	365	321	44	8.8
Ebenezer × Nebuka	220	92	128	101	27	12.3
Red Creole × Nebuka	24	8	16	14	2	8.3
Yellow Globe Danvers × Nebuka	50	14	36	31	5	10.0
Southport Red Globe × Nebuka	72	22	50	39	11	15.3
Sweet Spanish × Nebuka	23	18	5	5	0	0.0
White Portugal × Nebuka	220	54	166	103	63	28.6
Total	1,110	344	766	614	152	13.7
First backcross to *A. cepa*:						
Australian Brown × F_1	2	0	2	0	2	100.0
Early Grano × F_1	7	1	6	0	6	85.7
Southport Yellow Globe × F_1	4	0	4	0	4	100.0
Total	13	1	12	0	12	92.3
Second backcross to *A. cepa*:						
Early Grano × first backcross	49	11	38	25	13	26.5
Southport Yellow Globe × first backcross	21	0	21	3	18	85.7
White Portugal × first backcross	15	0	15	1	14	93.3
Total	85	11	74	29	45	52.9

backcross generations to *A. cepa* were as badly infected with smut as the original commercial varieties used in the crosses. Only 20 percent of the seedlings of the third backcross generation became infected below the crotch, but since only 25 seedlings were tested not much significance can be attached to this result.

TABLE 2.—*Results of testing commercial onion varieties, Nebuka, F_1 hybrid progenies, and first, second, and third backcrosses to onion for resistance to smut in the greenhouse at Madison, Wis., 1939–40*

Variety or pedigree	Plants tested	Plants not smutted	Plants smutted			Tested plants smutted below crotch
			Total	With lesion in indicated position		
				At or above crotch	Below crotch	
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
Allium cepa (commercial varieties):						
Brigham Yellow Globe	33	1	32	17	15	45.5
Crystal Wax	20	1	19	11	8	40.0
Early Yellow Globe	235	10	225	100	125	53.2
Mountain Danvers	159	15	144	58	86	54.1
Red Creole	57	1	56	12	44	77.2
Yellow Globe Danvers	145	8	137	18	119	82.1
Total	649	36	613	216	397	61.2
A. fistulosum (Nebuka)	1,088	936	152	146	6	0.6
F_1 (*A. cepa* × *A. fistulosum*):						
Crystal Wax × Nebuka	57	21	36	33	3	5.3
Red Creole × Nebuka	99	42	57	49	8	8.1
Mountain Danvers × Nebuka	39	8	31	25	6	15.4
Total	195	71	124	107	17	8.7
First backcross to *A. cepa*:						
Early Yellow Globe × F_1	200	10	190	59	131	65.5
White Portugal × F_1	12	0	12	4	8	66.7
Total	212	10	202	63	139	65.6
Second backcross to *A. cepa*:						
Brigham Yellow Globe × first backcross	38	3	35	20	15	39.5
Early Yellow Globe × first backcross	75	3	72	32	40	53.3
Southport White Globe × first backcross	16	0	16	3	13	81.2
Yellow Bermuda × first backcross	180	9	171	37	134	74.4
Total	309	15	294	92	202	65.4
Third backcross to *A. cepa*:						
Brigham Yellow Globe × second backcross	17	1	16	13	3	17.6
Early Yellow Globe × second backcross	8	0	8	6	2	25.0
Total	25	1	24	19	5	20.0

In the 1941–42 trials, Nebuka again proved highly resistant, only 0.5 percent of the plants developing lesions below the crotch as compared with 81.3 percent of the Yellow Globe Danvers and 82.1 percent of the backcross populations (table 3). Again, the backcross populations appeared as susceptible as the susceptible parent. A statistical analysis of the results summarized in table 3 failed to establish any significant differences between the various backcross populations in their degree of resistance to the smut organism.

In 1941 two populations of the amphidiploid were tested. One was a P_2 population obtained by selfing the original amphidiploid and the other a mixture of P_3 progenies from these P_2 plants. The data are summarized in table 4. None of the Nebuka plants smutted below the crotch, while 98.8 percent of the Yellow Globe Danvers became infected at an early stage. The amphidiploid was intermediate in its reaction, 38.0 percent of the P_2 and 59.1 percent of the P_3 population becoming infected below the crotch. While this difference between the P_2 and P_3 populations is highly significant, as measured by the chi-square goodness-of-fit test, it may be the result of variation in the quantity of the smut organism present rather than of a genetic difference between the P_2 and P_3 plants.

TABLE 3.—*Results of testing a commercial variety of onion, Nebuka, and backcrosses to onion for resistance to smut in the greenhouse at Madison, Wis., 1941–42*

Variety or pedigree	Plants tested	Plants not smutted	Plants smutted			Tested plants smutted below crotch
			Total	With lesion in indicated position		
				At or above crotch	Below crotch	
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
Allium cepa (Yellow Globe Danvers)	342	51	291	13	278	81.3
A. fistulosum (Nebuka)	189	181	8	7	1	.5
Backcross to *A. cepa:*						
Early Yellow Globe × (Brigham Yellow Globe×Nebuka)	71	9	62	3	59	83.1
Do	20	0	20	2	18	90.0
Do	220	32	188	17	171	77.7
Do	192	51	141	10	131	68.2
Brigham Yellow Globe×(Yellow Bermuda×Nebuka)	22	3	19	0	19	86.4
Do	111	30	81	4	77	69.4
Do	110	0	110	0	110	100.0
Do	131	16	115	10	105	80.2
Brigham Yellow Globe×(White Portugal×Nebuka)	26	3	23	3	20	76.9
Do	167	5	162	4	158	94.6
Do	107	5	102	4	98	91.6
Total for backcrosses	1,177	154	1,023	57	966	82.1

TABLE 4.—*Results of testing a commercial variety of onion, Nebuka, and P_2 and P_3 amphidiploid progenies for resistance to smut in the greenhouse at Madison, Wis., 1941*

Variety or amphidiploid population	Plants tested	Plants not smutted	Plants smutted			Tested plants smutted below crotch
			Total	With lesion in indicated position		
				At or above crotch	Below crotch	
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
Nebuka	479	468	11	11	0	0
Yellow Globe Danvers	166	0	[1] 166	2	57	[1] 98.8
Amphidiploid P_2 population	237	105	132	42	90	38.0
Amphidiploid P_3 population	734	77	657	223	434	59.1

[1] The position of the lesion on 107 of these plants was indefinite, largely because they were infected so severely that the first leaf stage was not reached. These plants were included with those smutted below the crotch in calculating the percentage smutted below the crotch.

The partial resistance of the amphidiploid was confirmed in a later test, summarized in table 5. Progenies from 15 P_2 amphidiploid plants were grown and the percentage of seedlings that became infected with smut below the crotch ranged from 6.5 to 26.1 percent, the mean infection being 17.2 percent. A statistical analysis showed that the differences in percentage of infection between these P_3 amphidiploid populations were not significant. From 60.0 to 76.9 percent of the seedlings of commercial varieties of onions were smutted below the crotch of the first leaf, while only 1.0 percent of the Nebuka seedlings were infected. The amphidiploid plants, therefore, were definitely intermediate in their reaction to smut infection.

TABLE 5.—*Results of onion smut resistance trials of Nebuka, commercial varieties of onion, and P_1 and P_3 amphidiploid populations, Madison, Wis., 1941–42*

Variety or amphidiploid number	Plants tested	Plants not smutted	Plants smutted			Tested plants smutted below crotch
			Total	With lesion in indicated position		
				At or above crotch	Below crotch	
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
White Portugal	168	17	151	26	125	74.4
Yellow Globe Danvers	70	15	55	13	42	60.0
Utah Sweet Spanish	39	4	35	5	30	76.9
Total	277	36	241	44	197	71.1
Nebuka	98	92	6	5	1	1.0
Amphidiploid No. 15	124	68	56	29	27	21.8
Amphidiploid No. 26	130	95	35	18	17	13.1
Amphidiploid No. 49	162	98	64	30	34	21.0
Amphidiploid No. 56	175	109	66	31	35	20.0
Amphidiploid No. 83	185	107	78	37	41	22.2
Amphidiploid No. 141	176	105	71	25	46	26.1
Amphidiploid No. 151	151	108	43	31	12	7.9
Amphidiploid No. 170	166	89	77	43	34	20.5
Amphidiploid No. 182	168	142	26	15	11	6.5
Amphidiploid No. 198	176	134	42	20	22	12.5
Amphidiploid No. 199	173	131	42	23	19	11.0
Amphidiploid No. 230	145	112	33	18	15	10.3
Amphidiploid No. 238	123	55	68	37	31	25.2
Amphidiploid No. 300	159	89	70	40	30	18.9
Amphidiploid No. 313	167	96	71	36	35	21.0
Total	2,380	1,538	842	433	409	17.2

DISCUSSION

It would seem that the most practical breeding procedure to follow in attempting to combine the smut resistance of the Nebuka parent with the desirable qualities of commercial varieties of *Allium cepa* is to make repeated backcrosses of the hybrids to the *A. cepa* parent. Since the F_1 plants show some resistance to the smut organism, indicating that smut resistance is partially dominant, it should be possible by testing the seedlings to select smut-resistant plants from second, third, and later backcross generations, although the results thus far have not proved very encouraging. Unfortunately, the F_1 and first backcross generations are self-sterile, but it is hoped that later backcross generations will show sufficient fertility to permit selfing, in order that homozygous fertile smut-resistant lines may be developed.

Since this backcrossing method has not thus far been very successful, it may be that the utilization of the fertile amphidiploid will prove to be more satisfactory. This amphidiploid has been successfully backcrossed to *Allium cepa*, to *A. fistulosum*, and to the diploid hybrid between these two species. Seed has been obtained from these triploid hybrids, but it is not possible to report on their reaction to smut until they have been tested for smut resistance.

Recently M. G. Toole and A. E. Clarke,[4] of this Division, have obtained tetraploid plants of both *Allium fistulosum* and *A. cepa* by treating germinating seeds with colchicine. These plants have been selfed successfully; also crosses have been made between them and the fertile amphidiploid.

Another method of attack which is being followed is backcrossing the diploid hybrid to *Allium cepa* and then getting fertility by doubling the chromosomes with colchicine. Work is being continued with this material in an effort to obtain smut-resistant commercial types.

SUMMARY

Seedlings of the Nebuka type of *Allium fistulosum* are highly resistant to the smut disease of onion, caused by the fungus *Urocystis cepulae*, while the commercial varieties of *A. cepa* are extremely susceptible. F_1 hybrids between these two species show considerably more resistance than the susceptible but not as much as the resistant parent. These hybrids are self-sterile, so that no F_2 populations were obtained. When backcrossed to *A. cepa* the backcross populations proved highly susceptible, but seedlings that survived the smut test have been retained for further breeding studies.

A fertile amphidiploid obtained by crossing these two species has shown considerable resistance.

LITERATURE CITED

(1) ANDERSON, P. J.
1921. DEVELOPMENT AND PATHOGENESIS OF THE ONION SMUT FUNGUS. Mass. Agr. Expt. Sta. Tech. Bul. 4: 99–133, illus.

(2) ———
1925. COMPARATIVE SUSCEPTIBILITY OF ONION VARIETIES AND OF SPECIES OF ALLIUM TO UROCYSTIS CEPULAE. Jour. Agr. Res. 31: 275–286.

(3) EMSWELLER, S. L., and JONES, H. A.
1935. AN INTERSPECIFIC HYBRID IN ALLIUM. Hilgardia 9: [265]–273, illus.

(4) EVANS, R. I.
1933. CYTOLOGICAL STUDIES ON THE PARASITIC RELATIONSHIP OF UROCYSTIS CEPULAE TO THE ONION. Amer. Jour. Bot. 20: 255–268, illus.

(5) ———
1937. CYTOLOGICAL STUDIES ON THE PARASITIC RELATIONSHIP OF UROCYSTIS CEPULAE TO ALLIUM FISTULOSUM. Amer. Jour. Bot. 24: 214–218, illus.

(6) FELIX, E. L.
1933. DISEASE RESISTANCE IN ALLIUM FISTULOSUM L. Phytopathology 23: 109–110.

(7) JONES, H. A., and CLARKE, A. E.
1942. A NATURAL AMPHIDIPLOID FROM AN ONION SPECIES HYBRID. Jour. Hered. 33: 25–32, illus.

---

[4] Unpublished data.

(8) LEVAN, A., VON.
1936. DIE ZYTOLOGIE VON ALLIUM CEPA × FISTULOSUM. Hereditas 21: 195–214, illus.

(9) ———
1941. THE CYTOLOGY OF THE SPECIES HYBRID ALLIUM CEPA × FISTULOSUM AND ITS POLYPLOID DERIVATIVES. Hereditas 27: 253–272, illus.

(10) MAEDA, T.
1937. CHIASMA STUDIES IN ALLIUM FISTULOSUM, ALLIUM CEPA, AND THEIR F_1, F_2 AND BACKCROSS HYBRIDS. Jap. Jour. Genet. 13: 146–159, illus.

(11) WALKER, J. C., and JONES, L. R.
1921. RELATION OF SOIL TEMPERATURE AND OTHER FACTORS TO ONION SMUT INFECTION. Jour. Agr. Res. 22: 235–262, illus.

(12) ——— and WELLMAN, F. L.
1926. RELATION OF TEMPERATURE TO SPORE GERMINATION AND GROWTH OF UROCYSTIS CEPULAE. Jour. Agr. Res. 32: 133–146, illus.

EFFECTS OF SOIL TREATMENTS ON THE GROWTH OF THE HIGHBUSH BLUEBERRY[1]

By T. A. Merrill[2]

Research associate horticulturist, Michigan Agricultural Experiment Station

INTRODUCTION

The cultivation of the blueberry is one of the newer fruit industries in North America. The blueberry plant is known to be profoundly influenced by soil, yet data are very limited on the influence of nutrient supply and fertilizer treatments on either vegetative growth or productivity. It is believed that the results of the study of various soil treatments reported in this paper may be useful to those engaged in the culture of this plant.

REVIEW OF LITERATURE

Coville,[3] who was the pioneer in the domestication and improvement of the highbush blueberry (*Vaccinium corymbosum* L.), showed that one of its principal requirements for satisfactory growth and productivity is a rather sharply acid soil. Data indicating, at least approximately, the pH tolerance of the plant were first reported in 1934 by Johnston.[4] According to Johnston there is a fairly wide range of pH within which blueberry plants will grow well. The optimum pH value was found to be approximately 4.4. The plants used in his experiments grew fairly well on a soil as acid as pH 3.4, which led to the belief that few Michigan soils are so highly acid that they are unsuitable for blueberry culture. However, poor growth, or even failure of plants, has been noted on certain extremely acid soils.

Beckwith and Coville,[5] reporting on the results of a blueberry fertilizer experiment in New Jersey, state: "An application of nitrate of soda at the rate of 250 pounds to the acre for two years did not materially increase the yield." However, they found that an application of a mixture of 250 pounds of nitrate of soda, 750 pounds of acid phosphate, and 250 pounds of 28 percent potash to the acre doubled the yield. They further state: "A mixture of 170 pounds of nitrate of soda, 230 pounds of dried blood, 340 pounds of steamed bone, 340 pounds of phosphate rock, and 170 pounds of 28 percent potash to the acre nearly tripled the yield." Beckwith and Coville draw the following conclusion: "The results indicate that fertilizer, if carefully mixed and used, will greatly increase the yield of blueberries."

---

[1] Received for publication March 31, 1943. Journal Article No. 670 (n. s.) from the Michigan Agricultural Experiment Station.

[2] The writer wishes to express his thanks to Director V. R. Gardner for making it possible to carry on this work and for help and suggestions as the work progressed; to Dr. W. D. Baten for his many helpful suggestions and for the plan of statistical analysis used in analyzing the data; to Prof. Stanley Johnston for assistance with the field work; to Dr. J. W. Crist for suggestions throughout the time the work was being carried on: to Dr. James E. Keefe, of Chicago and South Haven, Mich., and to William Reimer, of Grand Junction, Mich., for making it possible to carry on the field work.

[3] Coville, F. V. EXPERIMENTS IN BLUEBERRY CULTURE. U. S. Bur. Plant Indus. Bul. 193, 100 pp., illus. 1910.

[4] Johnston, S. THE CULTIVATION OF THE HIGHBUSH BLUEBERRY. Mich. Agr. Expt. Sta. Spec. Bul. 252, 52 pp., illus. 1934.

[5] Beckwith, C. S., and Coville, S. BLUEBERRY CULTURE. N. J. Agr. Expt. Sta. Cir. 229, 28 pp., illus. 1931.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 1
July 1, 1944
Key No. Mich.-43

Concerning the application of a nitrogen fertilizer on Saugatuck sand in Michigan, Johnston[6] states: "Sulphate of ammonia gave poor results, the plants producing somewhat less fruit than those receiving no fertilizer." Nitrate of soda failed to show any greater response than sulphate of ammonia. "Very good results followed the use of 335 pounds of superphosphate per acre. Good results also were obtained from the use of a 5–10–12 fertilizer at the rate of 335 pounds per acre." Johnston also found that doubling the amount of superphosphate (to 670 pounds per acre) gave an increased yield of 38.2 percent the first year and 23.4 percent the second year. These same plots, however, had received three previous applications of superphosphate at the rate of 335 pounds per acre. Evidence of the value of potash alone is lacking in Johnston's work, but he is of the opinion that it is beneficial because his largest yields were obtained from complete fertilizer plots. He concludes by saying: "* * * it is evident that another factor besides phosphorus was responsible for the gain. Since nitrogen alone was clearly not beneficial, potash can be considered as being responsible." Johnston also stated that the size of the fruit was larger on the complete fertilizer plots than on those that received superphosphate alone.

Doehlert and Shive,[7] in a study of the blueberry grown in sand cultural solutions, reported that the solutions giving the best results, both in plant growth and production of berries, were characterized by low monopotassium phosphate and high nitrogen content. They state: "The high-yielding group is also characterized by treatments in which high proportions of calcium nitrate and, generally, low proportions of ammonium sulfate predominate."

The results of Doehlert's and Shive's sand culture experiments were in close accord with the findings of Beckwith and Doehlert,[8] who demonstrated that the greatest yield of fruit was obtained with the use of nitrate of soda alone, though admitting that the benefit of a complete fertilizer may have been obscured by excessive applications of rock phosphate.

Doehlert and Shive[9] also found that the blueberry's need for magnesium was slight, 0.5 p. p. m., and increasing applications above that amount had a retarding effect upon growth.

Chandler and Mason,[10] referring to the upland sandy soils of Maine, state that increased growth and better color of foliage were obtained on all plots that received nitrogen, but the plants on the plots that received only phosphorus and potash were not much better than those on the untreated plots. Increased yields of fruit were obtained for three seasons from the plots that received nitrogen and for two seasons from those that received phosphorus and potash. The complete fertilizer gave an increase of 128.6 percent over the check for the first season, 85 percent for the second, and 217.6 percent for the third season. In summarizing their work, they state that fertilizers carrying nitrogen stimulated growth, increased the number of fruit buds per stem, and gave greater yields as compared with untreated plots.

---

[6] See footnote 4, p. 9.

[7] DOEHLERT, C. A., and SHIVE, J. W. NUTRITION OF BLUEBERRY (VACCINIUM CORYMBOSUM L.) IN SAND CULTURES. Soil Sci. 41: 341–350. 1936.

[8] BECKWITH, C. S., and DOEHLERT, C. A. FERTILIZER AND TILLAGE FOR BLUEBERRIES. N. J. Agr. Expt. Sta. Bul. 558, 8 pp. 1933.

[9] See footnote 7, above.

[10] CHANDLER, F. B., and MASON, I. C. Correspondence.

FIELD OBSERVATIONS

In the spring of 1932, a 14-acre field at Grand Junction, Mich., was set to highbush blueberries. The soil, a Saugatuck sand typical of those considered suitable for the growth of blueberries, had been well prepared for the plants, and tests for acidity and water-table determinations (12 to 36 inches) had revealed conditions favorable to growth. Nevertheless, 2 years after planting, many plants were missing, and by the spring of 1937 the the plants in an irregular area of 3 or 4 acres were dead (fig. 1). A thorough investigation of the soil was then made. The available nitrogen, phosphorus, potassium, and calcium content of the soil was as determined by Spurway's quick tests.[11] The data obtained (table 1) indicated that these nutrients were present in quantities sufficient for good plant growth.

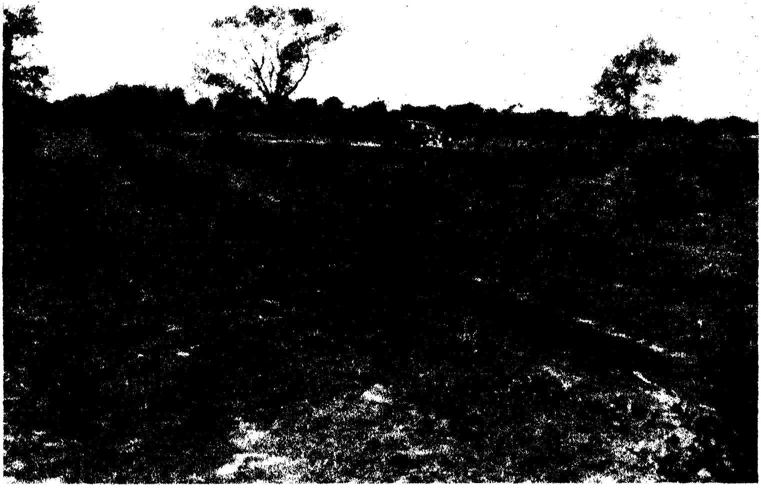


FIGURE 1.—In foreground s shown one of the high acidity areas in the experimental blueberry field at Grand Junction, Mich.; many plants are missing and others are making very poor growth. In upper left corner is an area that received two applications of ground limestone, each equivalent to 2,000 pounds per acre; plants are making very satisfactory growth with an abundant cover crop.

The pH values of the soil were extremely low (pH 3.2) in the areas where the plants were dead or dying (figs. 2, *A* and 4, *A*). However, inasmuch as Johnston [12] had grown plants successfully in soil with a pH value only slightly higher (3.4), this was not thought to be the cause of the poor growth and dying out of the plants, though from the soil tests (table 1, samples 1 and 2) the only apparent significant differences were in the pH values, and in the potassium and calcium content. It was therefore decided to undertake some greenhouse experiments and field trials in which various soil amendments would be used.

---

[11] SPURWAY, C. H. SOIL TESTING. A PRACTICAL SYSTEM OF SOIL DIAGNOSIS. Mich. Agr. Expt. Sta. Tech. Bul. 132, 16 pp. 1933.

[12] JOHNSTON, S. See footnote 4, p. 9.

TABLE 1.—*Soil analysis of a Saugatuck sand field, by the Spurway method; tests made in 1937*

Item	Sample 1 [1]	Sample 2 [2]	Sample 3 [3]
Reaction ... pH	3.2	4.4	3.8
Nitrates ... p. p. m.	5.0	5.0	20.0
Phosphorus ... p. p. m.	1.0	1.0	.5
Potassium ... p. p. m.	5.0	10.0	10.0
Calcium ... p. p. m.	20.0	75.0	40.0
Ammonia ... p. p. m.	2.0	2.0	
Iron	Trace	Trace	
Magnesium ... p. p. m.		Trace	5.0
Organic matter	High	High	High

[1] Soil taken from near plant shown in figure 4, *A*.
[2] Soil taken from area in field where plants were making normal growth.
[3] Soil taken from near plant shown in figure 4, *B*. Sample was taken a year after treatment was applied.

EXPERIMENT 1

POT CULTURES

A quantity of soil representative of the area in which the plants had died, was taken from the field, screened, and throughly mixed. Three and one-half gallon galvanized-steel pails, coated on the inside with an asphalt paint, were filled to the same weight with the soil. A single 1-year-old, well-rooted cutting of the Rubel variety of blueberry was set in each pail. Pails were filled to allow for three replications for each treatment listed in table 2. The pails were then placed in the greenhouse, where they remained from April 1, 1937 until the middle of the following October. They were then removed to an outside shed where they remained during the winter.

TABLE 2.—*Schedule of fertilizer treatments in pot-culture experiments*

Treatment No.	Fertilizer material applied [1]					
	N	P	K	Mg	Mn	Ca
1	N	P	K			
2	N	P	K	Mg	Mn	Ca
3		P	K	Mg	Mn	Ca
4	N		K	Mg	Mn	Ca
5	N	P		Mg	Mn	Ca
6	N	P	K		Mn	Ca
7	N	P	K	Mg		Ca
8	N	P	K	Mg	Mn	
9	N	P	K			Ca

[1] N-P-K applied as a 5-10-5 mixture at the rate of 500 pounds per acre, Mg (magnesium sulfate), 50 pounds per acre; Mn (manganese sulfate), 50 pounds per acre; Ca (Limestone), 2 tons per acre.

On March 1, 1938, the plants were returned to the greenhouse to remain throughout the growing season. The temperature in the greenhouse was held fairly uniform at a mean of about 70° F. until the early part of the summer and thereafter was allowed to vary with the outdoor temperature. The amount of water that the soil would hold was determined by the Hilgard cup method and the moisture content was then kept as uniform as possible at 90 percent of the determined capacity, which was approximately 80 percent of field capacity. This was done by bringing the pots up to weight daily with distilled water.

Limestone [13] was applied at the rate of a ton per acre and was thoroughly mixed with the soil before the pots were filled. The following spring a second application of a ton per acre was made and worked into the first inch or so of the soil. The other fertilizers were applied, after the buds started to break, to the surface of the soil to be carried down into it when watered.

---

[13] Limestone and fertilizer treatments were calculated on the basis of the area of the pots rather than on the weight of the soil in the pots.

The plants under treatment No. 1, in which magnesium, manganese, and limestone were withheld, and treatment No. 8, in which only limestone was withheld, started to grow normally and continued until about the first of July. At that time the tips of all the leaves on the terminal shoots began to show signs of burning. This burning was similar to that which had been observed under field conditions, beginning at the tips of the terminal leaves and finally progressing across the entire leaf. This condition developed progressively down the shoots to the older leaves, until by the latter part of August the plants were practically defoliated. All plants receiving limestone grew normally and showed no signs of either tip or marginal burning at any time during the growing season. The omission of magnesium or manganese, or both, had no noticeable effect upon the growth of the plants.

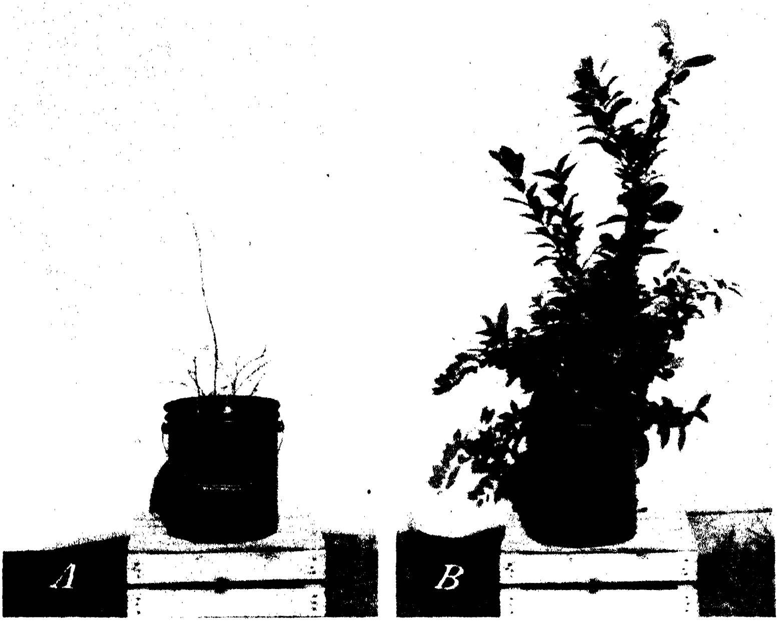


FIGURE 2.—Blueberry plant (check) that received one application of N-P-K mixture; pH of soil 3.2. *B*, Plant that received one application of N-P-K mixture plus two applications of ground limestone, equivalent to 2,000 pounds per acre per application; pH of soil 3.9 at time picture was taken 2 years after treatment.

Figure 2, *A* and *B*, shows plants from treatments 1 and 9 respectively (table 2). These plants received the same applications of nitrogen, phosphorus, and potash, but the plant shown in *B* received, in addition, ground limestone equivalent to 2 tons per acre in two applications, one at the time the soil was put into the pots, the other at the beginning of the second growing season. The soil reaction at the beginning of the experiment was pH 3.2; at the time the photograph was taken the pH of the soil in figure 2, *A*, had not changed but that in figure 2, *B*, had risen to 3.9. The plant shown in figure 2, *B*, was typical of all plants growing in soil to which limestone had been applied.

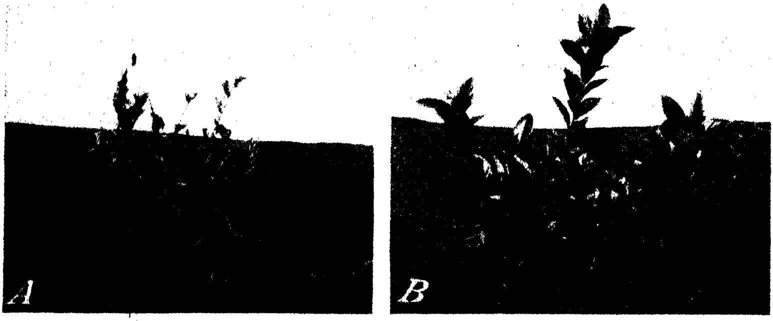


FIGURE 3.—*A*, Blueberry plant of Rubel variety growing in field given one application of muriate of potash; this plant is typical of the plants that received an application of muriate of potash either alone or in combination with nitrogen, phosphorus, or both. *B*, Rubel plant growing in check plot in the same area as the plant shown in *A*. Plants photographed the same day.

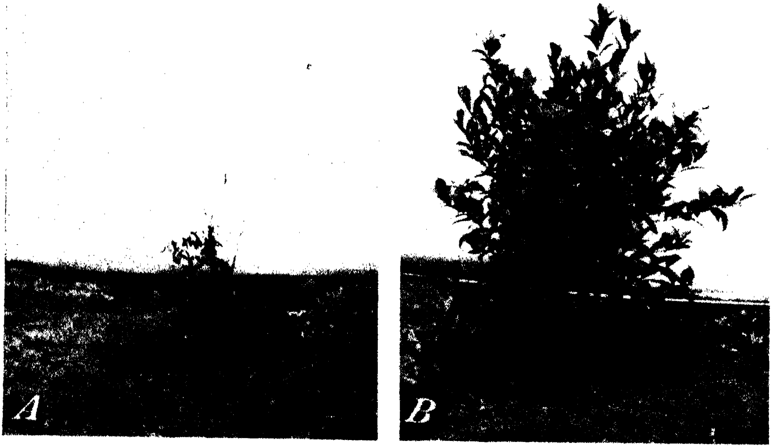


FIGURE 4.—*A*, Six-year-old plant growing under field conditions. *B*, Two-year-old plant growing in soil in the same area as the plant shown in *A*. Both plants received the same fertilizer application, but the plant shown in *B* received in addition two applications of ground limestone, each application equivalent to 2,000 pounds per acre.

Since nitrogen, phosphorus, and potash were present in this soil in sufficient quantities, the omission of these elements from the treatments (Nos. 3, 4, and 5) had no noticeable effect upon the growth of the plants.

FIELD TREATMENTS

In the field, the only fertilizer application made corresponded to treatment 9 in table 2. Two plots, each receiving the same treatment but in different locations, and each containing 110 plants (approximately one-tenth of an acre) were treated with a 5–10–5 fertilizer at the rate of 500 pounds per acre and an application of ground limestone at the rate of 1 ton per acre. The writer found under field conditions in Michigan that muriate of potash had a retarding effect, and in the majority of cases an injurious one, upon the plants. Figure 3, *A*, illustrates the effect of the muriate upon the plant. Figure 3, *B*, shows a plant that grew in the same area but received no fertilizer. The detrimental effect of muriate of potash was observed also by Collison[14] under conditions prevailing at Geneva, N. Y. Figure 4, *A*, shows a plant that was typical of those growing in the area where the plants were weak and dying. It is shown as it appeared July 26, 1938, in its sixth season of growth. The pH of the soil near this plant was 3.2, the same as the soil that was taken into the greenhouse and used in the pot cultures. Figure 4, *B*, shows a typical plant from the plots that had received two applications of ground limestone, each of 1 ton per acre. The pH of the soil near this plant had risen from 3.2 to 3.8. The two plants were photographed the same day. Although the plant in figure 4, *B*, was only in its second season of growth, it was much larger than the 6-year-old plant growing on the more acid soil.

EXPERIMENT 2

METHODS

The areas in Michigan that are most suitable for blueberry production consist of two types of soil, namely, Saugatuck sand and muck.[15] Both types were used in experiment 2.

A quantity of each soil was taken from the field into the greenhouse, where it was screened and thoroughly mixed. Three and one-half gallon galvanized-steel pails, as previously described, were filled with 47 pounds of the sand and 28 pounds of the muck. A single 2-year-old, well-rooted plant of the Rubel variety was set in each pail. A sufficient number of pails were filled with each soil to allow for four replications of each treatment, as follows:

Treatment No.	*Fertilizer*
1	No. fertilizer check.
2	Nitrate of soda (N).
3	Sulfate of ammonia (S).
4	Superphosphate.
5	Sulfate of potash.
6	Nitrate of soda + superphosphate.
7	Nitrate of soda + sulfate of potash.
8	Sulfate of ammonia + superphosphate.
9	Sulfate of ammonia + sulfate of potash.
10	Superphosphate + sulfate of potash.
11	Nitrate of soda + superphosphate + sulfate of potash.
12	Sulfate of ammonia + superphosphate + sulfate of potash.

---

[14] COLLISON, R. C. Unpublished data.

[15] These soils are hereafter referred to as sand and muck.

Throughout the experiment the plants were watered with distilled water and the pails were brought up to weight at each watering, thus eliminating as far as possible overwatering in some cases and underwatering in others. During the summer months the plants were kept out of doors in a frame house with open sides and an overhead protection of ordinary glass sash. During the winter months the roots were protected from excessive freezing by straw packed around the pails. The plants were grown in the pails throughout the first year without any fertilizer treatment in order to give all of them, insofar as possible, an equal start. At the beginning of the second growing season growth measurements were made and the plants were so arranged that the total growth of each of the groups of four plants which were to receive the different treatments varied no more than a few centimeters. Each group was then numbered from 1 to 12 and a fertilizer treatment assigned at random. The treatments were made on the basis of a 5–10–5 fertilizer applied at the rate of 500 pounds per acre. The second application was increased 50 percent over that of the previous season.

At the end of the third growing season, a sample of wood of the current season's growth was taken from each plant. As nearly as possible the same amount of wood was taken for each sample. This wood was cut into small pieces, placed in a drying oven and thoroughly dried at 70° C. It was then ground to pass through a 40-mesh sieve. Each ground sample was placed in a tightly stoppered bottle and was later analyzed for N, P_2O_5, and K_2O.

After the samples were taken for chemical analysis, the plants were cut off at the soil surface and the tops were thoroughly dried. The dry weights were then recorded.

Tables 3 and 4 present the complete data of the experiments, the former those for the dry-matter weight of the tops, and the latter, the percent of nitrogen, phosphoric acid and potash in the wood.

TABLE 3.—*Total oven-dry weight of top growth of 4 blueberry plants in 2 soils and in 12 fertilizer treatments*

SAND SOIL

Replication No.	Weight of top growth following fertilizer treatment No. —[1]											
	1	2	3	4	5	6	7	8	9	10	11	12
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	250	256	222	256	159	279	243	328	214	195	328	324
2	233	305	301	285	226	342	315	308	210	288	348	356
3	233	262	274	226	270	315	314	266	268	209	313	347
4	238	163	280	192	279	315	222	298	237	291	315	344
Average	238	246	269	240	234	313	273	300	232	246	326	343

MUCK SOIL

1	260	365	402	212	215	317	381	342	398	276	404	373
2	390	372	348	191	303	437	303	348	414	248	468	477
3	207	320	203	189	240	450	405	370	445	235	432	363
4	281	395	318	244	256	375	375	366	363	287	521	475
Average	284	363	318	209	253	395	366	356	405	261	456	422

[1] For explanation of fertilizer treatments, see text.

TABLE 4—*Chemical analysis of wood of 4 blueberry plants in 2 soils and in 12 fertilizer treatments*[1]

Replication No.	Analysis of wood of blueberry plants following fertilizer treatment No.—[2] [3]											
	1	2	3	4	5	6	7	8	9	10	11	12
	N IN PLANTS GROWN ON SAND SOIL											
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	0. 617	0. 924	0. 834	0. 548	0. 709	0. 701	0. 789	0. 737	0. 836	0. 651	0. 859	0. 559
2	. 490	. 842	. 651	. 627	. 611	. 716	. 805	. 761	. 770	. 576	. 688	. 697
8	. 614	. 708	. 661	. 571	. 551	. 657	. 765	. 696	. 701	. 764	. 894	. 723
4	. 598	. 668	. 869	. 472	. 635	. 776	. 747	. 763	. 791	. 620	. 730	. 714
Average	. 580	. 785	. 754	. 554	. 626	. 712	. 776	. 739	. 774	. 652	. 634	. 673
	N IN PLANTS GROWN ON MUCK SOIL											
1	0. 521	0. 621	0. 643	0. 523	0. 564	0. 415	0. 667	0. 660	0. 716	0. 567	0. 688	0. 585
2	. 594	. 631	. 670	. 408	. 495	. 661	. 549	. 635	. 618	. 538	. 668	. 577
3	. 487	. 663	. 891	. 578	. 599	. 598	. 743	. 583	. 692	. 521	. 562	. 604
4	. 567	. 661	. 743	. 616	. 465	. 643	. 592	. 647	. 620	. 559	. 617	. 607
Average	. 542	. 644	. 736	. 531	. 531	. 579	. 638	. 631	. 661	. 546	. 634	. 593
	P_2O_5 IN PLANTS GROWN ON SAND SOIL											
1	0. 120	0. 138	0. 126	0. 174	0. 139	0. 195	0. 101	0. 223	0. 106	0. 205	0. 232	0. 117
2	. 118	. 168	. 109	. 212	. 157	. 171	. 099	. 227	. 102	. 217	. 229	. 214
3	. 120	. 092	. 102	. 181	. 102	. 216	. 112	. 190	. 117	. 237	. 229	. 234
4	. 135	. 083	. 149	. 223	. 122	. 202	. 117	. 218	. 117	. 196	. 233	. 194
Average	. 123	. 120	. 121	. 197	. 130	. 196	. 107	. 214	. 110	. 214	. 231	. 190
	P_2O_5 IN PLANTS GROWN ON MUCK SOIL											
1	0. 110	0. 095	0. 118	0. 200	0. 112	0. 163	0. 105	0. 234	0. 078	0. 238	0. 229	0. 265
2	. 123	. 109	. 096	. 154	. 096	. 243	. 103	. 228	. 091	. 228	. 274	. 253
3	. 087	. 200	. 113	. 186	. 115	. 209	. 106	. 223	. 103	. 188	. 226	. 245
4	. 117	. 088	. 109	. 215	. 080	. 187	. 127	. 230	. 096	. 216	. 247	. 251
Average	. 109	. 123	. 109	. 189	. 101	. 200	. 110	. 229	. 092	. 217	. 244	. 253
	K_2O IN PLANTS GROWN ON SAND SOIL											
1	0. 325	0. 253	0. 265	0. 345	0. 590	0. 250	0. 398	0. 283	0. 612	0. 611	0. 488	0. 450
2	. 265	. 291	. 306	. 355	. 536	. 233	. 376	. 317	. 434	. 744	. 468	. 546
3	. 363	. 213	. 320	. 414	. 508	. 318	. 424	. 326	. 470	. 686	. 471	. 555
4	. 302	. 197	. 289	. 477	. 608	. 325	. 259	. 267	. 541	. 606	. 447	. 452
Average	. 314	. 238	. 295	. 398	. 560	. 281	. 364	. 298	. 514	. 662	. 468	. 501
	K_2O IN PLANTS GROWN ON MUCK SOIL											
1	0. 317	0. 263	0. 266	0. 393	0. 333	0. 230	0. 485	0. 236	0. 426	0. 519	0. 467	0. 559
2	. 296	. 285	. 280	. 251	. 438	. 293	. 477	. 256	. 454	. 491	. 533	. 429
3	. 270	. 267	. 308	. 377	. 344	. 253	. 436	. 245	. 390	. 403	. 432	. 496
4	. 294	. 334	. 284	. 333	. 263	. 297	. 414	. 301	. 466	. 500	. 499	. 445
Average	. 294	. 287	. 284	. 338	. 344	. 268	. 453	. 259	. 434	. 478	. 483	. 482

[1] Analysis made by Dr. E. J. Miller and staff, Department of Chemistry, Michigan Agricultural Experiment Station.

[2] For explanation of fertilizer treatments see text.

[3] Percentages given are on dry-weight basis.

RESULTS

The experiments were so designed that Fisher's method of analysis of variance could be used to test significance, and this analysis was made. The use of fertilizer treatments in general gave very significant effects on the growth of the blueberry plants in both Saugatuck sand and muck soil. In a further analysis made to determine which particular element or combination of elements was responsible for the increased growth, nitrogen and phosphorus were found most effective when applied to the sand and nitrogen and potash when used on the muck. The effect of applications of nitrogen and phosphorus on total growth was significant at the 1-percent point for the plants growing in the sand; potash had no apparent beneficial effect.

The rank correlation between nitrogen accumulation in the wood and total growth of blueberry plants in the sand was 0.90 ± 0.06 and between phosphorus and total growth, 0.75 ± 0.13, while for potash it was 0.18 ± 0.28. The corresponding rank correlation coefficients for plants grown in the muck were: Nitrogen, 0.93 ± 0.04; phosphorus, 0.63 ± 0.18; and potash, 0.87 ± 0.07. There is a direct relation between total growth and accumulation of nitrogen and phosphorus in the wood of plants grown in the sand and between total growth and nitrogen and potash content of plants grown in the muck. Inasmuch as potash did not benefit the plants, from the standpoint of total growth in the sand, and phosphorus did not benefit the plants in the muck, the accumulation of these two elements in the wood of the plants on those soils could be classed as "luxury consumption."

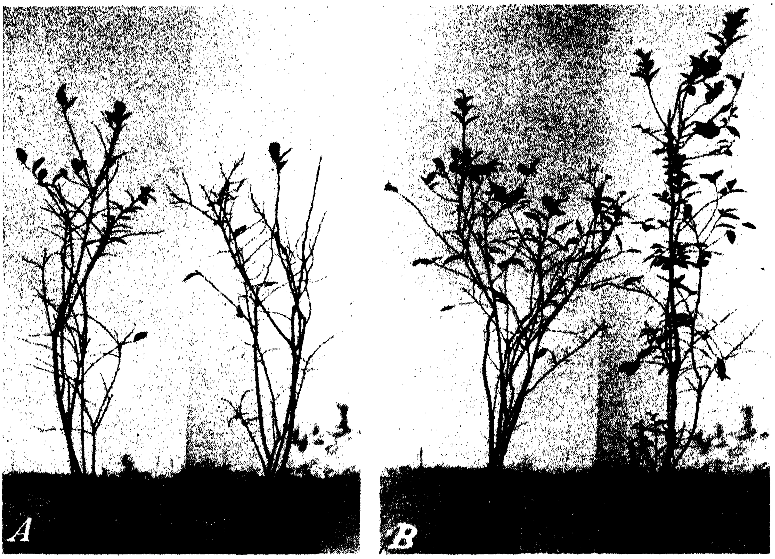


FIGURE 5.—*A*, Plants growing in sand with no fertilizer (check); *B*, plants growing in sand with complete fertilizer. The check plants matured earlier than those receiving the complete fertilizer and the lateral branches on the shoots were not so extensive. Photographing delayed until defoliation was almost complete so as to give a better conception of the character of growth.

The analysis of the data indicated that the application of neither phosphorus, potash, nor, a combination of the two increased the percentage of nitrogen in the wood. Further, neither nitrogen nor potash applications nor their combination increased the percentage of phosphorus in the wood. Nitrogen applications did exert a significant effect on the accumulation of potash in the wood of plants grown on the sand, but no significant effect on plants grown on the muck.

DISCUSSION

Observation and experience have demonstrated that in the main two types of soil, namely, muck or peat and sandy muck or sand containing a rather high percentage of organic matter, are suitable for blueberry culture; further, that blueberry plants thrive naturally in both types of soil, that in both types there is considerable variation in amount of growth (figs. 5 and 6), and that, in general, productivity and yields follow fairly closely the amount of growth and the size of plant.

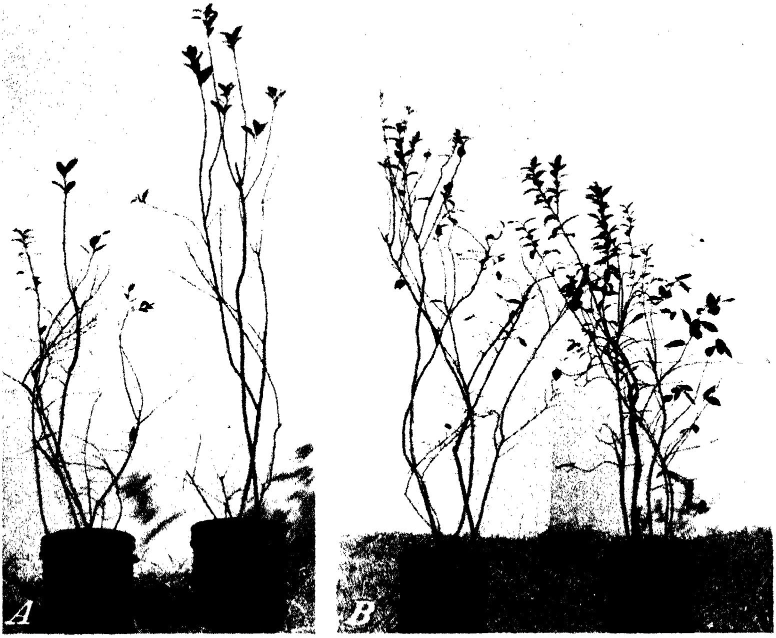


FIGURE 6.—*A*, Plants growing in muck with no fertilizer (check); *B*, plants growing in muck with complete fertilizer. The check plants matured eariler than those receiving the complete fertilizer and growth was more spindly and sparse. Photographing delayed until defoliation was almost complete so as to give a better conception of the character of growth.

What appeared to be average or typical soils of these two types were employed in this study. In composition, the soils differ greatly from each other as the muck or peat is of organic origin while the sand or sandy muck is of mineral origin though containing much organic material. This study corroborates the findings of others in showing that the blueberry plant is very sensitive to differences in soil acidity when the lower limits of tolerance are approached, and indicates that attention to soil reaction is of first importance when plants are not making normal growth.

This study also shows that available nutrients may be a limiting factor of secondary importance, though it does not point to any one nutrient element as most likely to be lacking or deficient in either a muck or a sandy muck soil. Unless or until there is definite evidence in any patricular case that some one nutrient element is the limiting factor the only safe course is to use a complete fertilizer mixture, consisting of nitrogen, phosphorus, and sulfate of potash.

SUMMARY AND CONCLUSION

In the soil under test, pH 3.2 was below the limit for vigorous growth of blueberry plants.

Symptoms associated with too high acidity are leaf scorch, which begins at the leaf margins and progresses across the leaf, resulting in defoliation. This may be followed by stunting and finally by death of the plants.

Applications of lime sufficient to raise the pH of th soil from 3.4 to 3.8 prevented leaf scorch and resulted in normal growth.

A positive correlation was found between total growth of plants and total milligrams of nitrogen and phosphorus in the tops of the plants grown in the sand and between total growth of plants and nitrogen and potash content of plants grown in the muck.

Muriate of potash should be omitted from the complete fertilizer mixture.

POTATO VARIETIES IN RELATION TO BLACKENING AFTER COOKING[1]

By G. H. RIEMAN, *professor of genetics, horticulture, and plant pathology*, W. E. TOTTINGHAM, *associate professor of biochemistry*, and JOHN S. MCFARLANE, *instructor in genetics and economic entomology*, *Wisconsin Agricultural Experiment Station*

INTRODUCTION

Blackening after cooking is regarded as one of the commonest defects of the potato (*Solanum tuberosum* L.). Spangler (*10*) [2] found that 7 percent of 1,165 Chicago retailers listed tendency to "cook black" as the most serious defect of potatoes and nearly 18 percent listed it as the second most objectionable defect. Hotchkiss et al. (*3*) report cooking black as the commonest complaint of 880 retailers in the cities of Cleveland and Rochester.

During the past 40 years numerous studies have been made on the relation of conditions of growth to darkening in potato tubers. However, the control of this defect is still an important problem in many potato-producing regions. Ashby (*1*), working in England, noted a marked influence of climatic factors, such as seasonal rainfall and air temperature, on the ability of various types of soil to produce potatoes free from darkening. Other investigators have observed similar climatic effects. Tottingham, Nagy, and Ross (*11*) reported that blackening was particularly serious in crops produced during growing seasons characterized by excessive heat and drought. Nash and Smith (*6*) suggested that lack of sufficient sunlight and low temperatures may be important in influencing the occurrence of blackening. More recently Smith, Nash, and Dittman (*9*) observed that tubers maturing under low temperatures ranging from 50° to 60° F. were likely to blacken, whereas those maturing at higher temperatures ranging from 70° to 80° seldom showed discoloration. They also noted that high storage temperatures for 3 to 4 days prevented blackening.

Nutritional factors in relation to blackening have received considerable attention from American and European investigators during the past 15 years. Although these studies have made many valuable contributions to the knowledge of the nutritional requirements of the potato plant, they have not led to a definite understanding of the causes of blackening in cooked potatoes. As a matter of fact, it has not been possible thus far to induce blackening with certainty under controlled experimental conditions by varying any environmental factor.

Varietal response to blackening has received but little attention from workers interested in this problem. Parker (7) stated that color after cooking is influenced by soil rather than variety. Nash

---

[1] Received for publication April 12, 1943. Contribution from the Departments of Genetics (paper No. 313), Horticulture, Plant Pathology, Biochemistry, and Economic Entomology, Wisconsin Agricultural Experiment Station. Photographs taken by Eugene H. Herrling, Department of Plant Pathology, Wisconsin Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 31.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 1
July 1, 1944
Key No. Wis.-145

(*5*) found that Green Mountain, Pioneer Rural, and Sebago tubers blackened most seriously, and Houma, Pontiac, and Chippewa blackened least.

The object of the present study was to examine certain possible relation ships between the genetic constitution of the potato plant and the blackening character frequently observed in cooked potato tubers.

PLANT MATERIALS AND METHODS

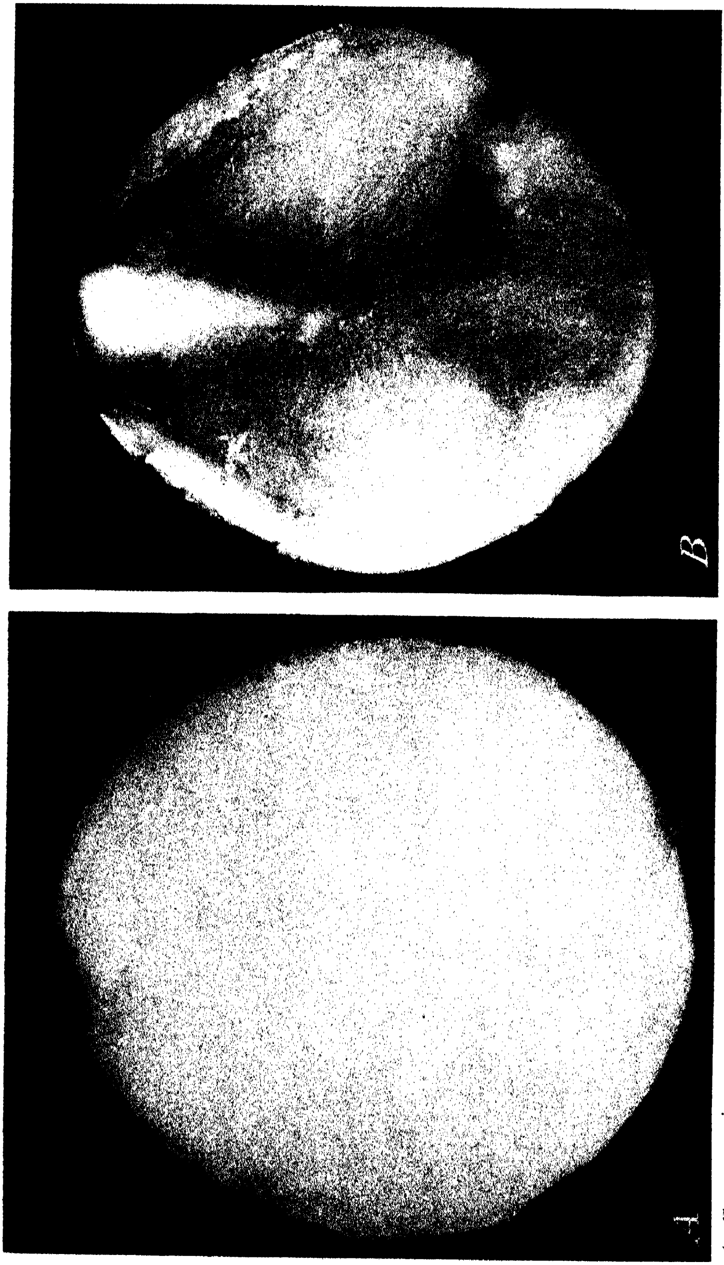
Twenty-three varieties and strains of potatoes were grown in nine widely separated locations in Wisconsin during the 5-year period 1937–41. These test plots were located on four general soil types, as follows: Three on clay loam, two on sandy loam, two on silt loam, and two on muck. The seed, for the most part, was produced in tuber-unit plots in the extreme northern part of the State, placed in suitable common storage, and divided equally for the various test plots. The test plots were planted during the last 2 weeks in May and the first week in June. They were harvested during the last 2 weeks in September and the first week in October. All of the varieties at a given location were planted at the same time and harvested at the same time. Four 25- or 50-hill single-row randomized replicates of each variety were grown at each location. Yield records were taken on each replicate. A composite sample for boiling tests was drawn from the four replicates and placed in common storage at about 42° F. The boiling tests were made approximately 2 months after harvest. Nine hundred sixty-eight boiling tests were made during the course of this study.

The essential features of the cooking method were held constant for the 5-year period of the experiment. Ten-tuber samples of fairly uniform size were washed and cooked with the skins intact during the first 2 years, but during the last 3 years the samples were peeled and washed in an abrasive machine. The tubers were cut in halves longitudinally and placed in uniform aluminum cooking vessels containing similar amounts of cold tap water. The usual kitchen method of trial with a knife or fork was used to determine when the potatoes were cooked. Obvious errors in judgment in the cooking process were corrected by boiling another sample. The cooked halves were placed flat face upward on filter paper and allowed to cool for approximately a half hour at room temperature before color readings were made. The flat cut surfaces, exposing longitudinal sections through the center of the tubers, were used to determine the amount of blackening. Color readings were made under a fluorescent lamp in a compartment painted black internally. The tubers in a sample were classified as follows: 1=white; 2=light gray; 3=medium gray; 4=dark gray, as indicated in plate 1. Photographs showing the amount of blackening allowed for each class were used as standards.

Blackening indices were calculated for each variety grown at each location whereby equal weight was given to the four blackening classes and a range established from 0 for all white tubers to 100 for all dark-gray tubers.[3]

---

[3] Blackening index=Number of tubers in classes 1, 2, 3, 4 multiplied by 0, 1, 2, 3, respectively. Sum of these products divided by the product of 3 times the number of tubers. This quotient multiplied by 100=Blackening index.



A, Cross section through a Triumph potato tuber showing no blackening one-half hour after boiling. Classification, white or class No. 1. *B*, Cross section through a Russet Rural potato tuber showing considerable blackening one-half hour after boiling. Classification, medium gray or class No. 3.

An attempt was made to determine the optimum stage for the expression of blackening in stored potatoes. The least amount of blackening after boiling was observed at the time of harvest while the greatest amount was noted in the spring after 5 months storage when the tubers were soft and heavily sprouted. Classification of blackening after boiling became progressively more variable as sprouting activity increased. The most uniform results in measuring the amount of blackening—as judged by duplicate 10-tuber tests—were obtained on dormant tubers held in storage at 42° F. for a period of approximately 2 months.

RESULTS

VARIETIES AND STRAINS IN RELATION TO BLACKENING

A study of the data in table 1 shows that although blackening was markedly affected by seasonal growing conditions, the general order of varietal response was maintained during the 5-year period of the test. Among the first nine varieties which have been tested each year, Triumph and Chippewa exhibited definite tendencies to cook white with low 5-year average blackening indices of 20 and 22, respectively. Rural New Yorker and Russet Rural showed a consistent tendency to cook dark with high 5-year average blackening indices of 46 and 49, respectively, or about twice the amount of blackening observed in Triumph and Chippewa. The remaining five varieties in this group—Houma, Katahdin, Red Warba, Irish Cobbler, and Green Mountain—showed intermediate reactions with blackening indices ranging from 29 to 41. The six varieties in the second group which were tested only during the first three seasons—Earlaine, 100 Day Cobbler, Pioneer Rural, Mesaba, Russet Burbank, and Columbia Russet—all produced considerable amounts of blackening. The four varieties in the third group which were tested during different seasons—White Blossom Cobbler, Sebago, Pontiac, and Sequoia—produced various amounts of discoloration.

TABLE 1.—*Blackening after boiling of tubers of 19 potato varieties and strains grown in 9 Wisconsin counties*

Variety or strain	Average blackening indices for cooked samples[1]						
	1937	1938	1939	1940	1941	3-year average 1937–39	5-year average 1937–41
Triumph	57	17	11	6	9	28	20
Chippewa	57	30	9	8	7	32	22
Houma	67	30	20	19	9	39	29
Katahdin	52	33	30	23	13	38	30
Red Warba	76	38	7	13	14	40	30
Irish Cobbler	76	63	26	10	17	55	38
Green Mountain	71	67	32	21	14	57	41
Rural New Yorker	81	59	37	31	24	59	46
Russet Rural	83	70	37	35	21	63	49
Earlaine	72	33	24			43	
100 Day Cobbler	77	56	39			58	
Pioneer Rural	76	56	39			57	
Mesaba	81	56	39			59	
Russet Burbank	67	67	33			56	
Columbia Russet	76	75	30			60	
White Blossom Cobbler	81	63					
Sebago		11	13	4	6		
Pontiac			17	13	14		
Sequoia					16		

[1] Blackening index: 0=white ½ hour after boiling; 100=dark gray ½ hour after boiling.

Of the 19 varieties listed in table 1, 6 were early-maturing, 2 were midseason, and 11 were late-maturing. Varieties with a tendency to cook white and varieties with a tendency to cook dark were observed in all 3 maturity classes.

Clonal selections derived from standard varieties behaved like the parental stocks. Pioneer Rural, a New York clonal selection out of Russet Rural, produced similar amounts of blackening during three seasons. Another New York clonal selection under test for three seasons, 100 Day Cobbler out of Irish Cobbler, behaved like its parental variety. Columbia Russet, a periclinal chimera apparently derived from Green Mountain, produced blackening in amounts similar to Green Mountain over a 3-year period. The Minnesota periclinal chimera Red Warba, out of the pink-eyed Warba, produced similar results during one season. White Blossom Cobbler cooked like Irish Cobbler for 2 years, although in this case a clonal relationship between the two varieties has not been clearly established. The periclinal chimera Russet Rural, out of Rural New Yorker, developed similar amounts of blackening during the 5-year period of the experiment. Four varieties and strains which were tested for only 1 year at the nine locations have been omitted from table 1. These include the New York Rural strain Toanco No. 4, the Wisconsin Rural strain Martin Seedling, the New York Irish Cobbler strain Cottrell Cobbler, and the Minnesota variety pink-eyed Warba already mentioned. The behavior of Toanco No. 4 and Martin Seedling was similar to that of the two standard Rural varieties. Cottrell Cobbler developed the same amount of blackening after cooking as the standard Irish Cobbler variety.

The new Sebago variety tended to produce white-cooking tubers for a 4-year period; the new variety Pontiac tended to produce intermediate-colored tubers in cooking tests for 3 years. These results indicate that breeding for varieties having a tendency to cook white may be accomplished since the parents of these varieties, Chippewa, Katahdin, and Triumph, all have favorable cooking records.

HYBRID POPULATIONS IN RELATION TO BLACKENING

Additional evidence on the mode of inheritance of blackening after cooking is presented in table 2. Two F_1 populations resulting from crosses between the Sebago or Katahdin varieties, which tend to cook white, with the Hindenburg variety, which tends to cook dark, were tested for one season according to the procedure previously described. Normal-sized second clonal generation tubers were planted in Bayfield County during the middle of June 1941 and harvested while the vines were still green during the second week in October. Less than 1 percent of the vines were mature at harvest time. Two hundred and sixty-seven F_1 individuals were classified on the basis of approximately 5 tubers per cooked sample in place of the customary 10-tuber samples. The distributions of the 2 F_1 populations resulting from Sebago × Hindenburg and Katahdin × Hindenburg were similar. The F_1 populations were extremely variable. An array of F_1 individuals ranged throughout the blackening scale, which exceeded the limits observed for the 3 parental varieties. Seventy-six percent of the segregates from the 2 crosses resembled the 2 parents which tend to cook white, 14 percent developed the same amount of blackening as the dark-cooking parent, and 10 percent exhibited more blackening than the dark-cooking parent.

TABLE 2.—*Blackening after boiling of tubers of 3 parental potato varieties and 2 F_1 populations*

Stock	Segregates	Total tubers	Blackening classes[1]			
			White to light gray (BI range, 0–24)	Light gray (BI range, 25–49)	Medium gray (BI range 50–74)	Dark gray (BI range, 75–100)
	Number	*Number*				
Sebago		10	1			
Katahdin		10		1		
Hindenburg		10			1	
Sebago × Hindenburg	131	735	51	48	20	12
Katahdin × Hindenburg	136	791	58	45	18	15

[1] BI = Blackening index.

LOCATION IN RELATION TO BLACKENING

The results of cooking tests conducted over a period of 5 years on nine varieties grown in nine potato-producing counties in Wisconsin are presented in table 3. All locations produced large amounts of blackening during the hot, dry growing season of 1937 and small amounts during the cool, wet growing seasons of 1940 and 1941. However, the maturing and harvesting seasons during the last 2 weeks in September and the first week in October were cooler in 1937 than in 1940 and 1941. The southern stations in general produced somewhat less blackening than the northern stations. For potatoes grown on muck soil in southern Walworth County the average blackening index for the 5-year period was 25, while for the potatoes grown on sandy loam soil in Oneida County at a higher elevation and approximately 200 miles farther north, the average blackening index was 40.

TABLE 3.—*Blackening after boiling of tubers of 9 potato varieties grown in 9 Wisconsin counties*

County and soil series	Blackening index[1] in—				
	1937	1938	1939	1940	1941
Walworth: Muck[2]	56	41	17	9	1
Washington: Miami clay loam	66	44	13	17	7
Door: Miami loam	74	48	22	17	8
Langlade: Antigo silt loam	78	63	20	13	30
Oneida: Vilas sandy loam	85	41	28	28	19
Waushara: Waukesha sandy loam	59	56	33	26	13
Portage: Muck[2]	62	48	15		21
Kenosha: Carrington clay loam		42	20	15	5
Barron: Spencer silt loam		22	39	22	21
Average	69	45	23	18	14

[1] Averages for 9 varieties: 0 = white ½ hour after boiling; 100 = dark gray ½ hour after boiling.
[2] Soil not classified.

SEASON IN RELATION TO BLACKENING

The data in table 3 show a definite seasonal influence on blackening. A high average blackening index of 69 was recorded in 1937 for nine varieties at seven locations. Lower blackening indices were recorded for the same nine varieties during each succeeding year extending to 1941, when the average blackening index dropped to 14 for nine locations. During the 5-year period of this experiment the first two seasons represented heavy blackening years in Wisconsin while the last three seasons represented nonblackening years. These results agree with the experience of the trade with stored potatoes during the 1937 to 1941 period. Beginning with the 1939 crop, a minor change was made in the cooking technique which may account for some, but not all, of the difference in blackening observed between the first 2 years and the last 3 years of the experiment. In 1937 and 1938 the tubers were boiled with the skins intact while in the remaining 3 years they were washed and partly peeled with an abrasive machine.

A series of correlation studies was made on the effect of temperature, precipitation, and cloudy days during the five growing and harvesting seasons on the occurrence of blackening. Weather records collected by official weather stations located near each of the nine test plots and published by the United States Department of Commerce were used in this study. No significant associations were observed between either precipitation or cloudy days and the incidence of potato blackening. The associations between mean monthly air temperatures during the growing and harvesting period and the occurrence of blackening are of unusual interest. A nonsignificant low negative correlation was found when the mean air temperatures for the 4 months June, July, August, and September were averaged. However, when the mean air temperatures for single months were considered, two significant negative correlations and one significant positive correlation were found between air temperature and occurrence of blackening (table 4)

TABLE 4.—*Correlations between mean monthly air temperatures during the months of the growing and harvesting season and the occurrence of blackening in potatoes*

Month	*r* value	*P* value
June	−0.5	0.01
July	−.2	.50
August	+.4	.02
September	−.5	.01
June, July, August, September	−.2	.50

DISCUSSION AND CONCLUSIONS

Potato blackening after cooking is attributed by Merkenschlager (*4*) to an accumulation of tyrosine and unusual activity of tyrosinase. Tottingham, Nagy, and Ross (*11*) and Ross and Tottingham (*8*) have observed an association of the blackening abnormality with instability of protein and greater activity of the tyrosinase system in the potato tuber. The presence of an activator or activators of the oxidation of tyrosine was found in tubers which discolored after cooking and was absent from tubers which remained white after cooking. The amount of a substance or substances giving the catechol reaction in potatoes after about 3 months in cold storage has been found by Clagett and Tottingham (*2*) to be correlated to a considerable extent with the degree of blackening after boiling. Such studies have shown that these biochemical relationships, responsible in part at least for the occurrence of blackening in cooked tubers, are greatly influenced by conditions of growth and storage. That they may also be profoundly influenced by the genetic constitution of the potato plant is demonstrated in the present study.

GENETIC INFLUENCES

Genetic differences responsible for variations in the development of dark pigments in cooked tubers are common among American cultivated potato varieties. This is shown by the wide differences observed among nine varieties selected at random for blackening studies. Of these nine varieties which were tested for five seasons at nine widely separated locations, the two whitest-cooking varieties, Triumph and Chippewa, produced on an average only about one-half as much blackening as the two darkest-cooking varieties, Russet Rural and Rural New Yorker. Fourteen additional varieties have been tested in a similar manner for from one to four seasons. The new Sebago variety included in this group maintained an exceptionally white-cooking record over a 4-year period, but no variety observed was immune to blackening after cooking. A general grouping of 22 varieties and strains may be made in 3 classes as shown in table 5.

An examination of the varieties listed in these three classes in respect to maturity indicates that this variable was relatively unimportant in the development of black pigment since both late- and early-maturing varieties were observed in all three classes. It is of interest to note here that the three varieties listed in the white-cooking class present an unusually wide range in maturity. In this study, Triumph was consistently classified as a first early variety. As a rule, Chippewa was classified as a midseason variety, although in a number of cases it was inclined to be late. In all cases, Sebago was classified as an extremely late variety.

TABLE 5.—*General grouping of 22 varieties and strains of potatoes according to their tendency to cook white, intermediate, or gray*

Tendency to cook white	Tendency to cook intermediate	Tendency to cook gray
Triumph	Houma	Irish Cobbler
Chippewa	Katahdin	Cottrell Cobbler
Sebago	Red Warba	100 Day Cobbler
	Warba	White Blossom Cobbler
	Earlaine	Green Mountain
	Pontiac	Columbia Russet
		Rural New Yorker
		Russet Rural
		Pioneer Rural
		Martin Seedling
		Toanco No. 4
		Mesaba
		Russet Burbank

Eight, and possibly nine, of the 22 varieties listed in table 5 represent clonal selections out of Rural New Yorker, Irish Cobbler, Green Mountain, and Warba varieties. Three of the clonal selections appear to be identical with their parental varieties while the others differ from their parents in one or more plant characteristics. Four of them are periclinal chimeras. Since the clonal selections were probably made on the basis of characteristics other than cooking quality and since it is known that their genetic constitutions are similar to those of their parents, they should react like their parents in cooking tests.

The parent-offspring comparisons in table 6 clearly show that this is the case.

TABLE 6.—*Comparison of the cooking qualities as indicated by average blackening indices, of parental varieties of potatoes and their clonal selections*

Years tested	Parental variety	Average blackening index	Clonal selection	Average blackening index
1939	Warba	10	Red Warba	7
1937	Irish Cobbler	76	Cottrell Cobbler	86
1937-38	do	70	White Blossom Cobbler [1]	72
1937-39	do	55	100 day Cobbler	58
1937-41	Rural New Yorker	46	Russet Rural	49
1937-39	do	59	Pioneer Rural	57
1937	do	81	Martin Seedling	81
1937	do	81	Toanco No. 4	78
1937-39	Green Mountain	57	Columbia Russet	60

[1] Clonal relation to Irish Cobbler not clearly established.

The fairly close agreements between the clonal selections and the parental varieties from which they were derived not only demonstrate the relative unimportance of clonal strains in respect to blackening but they also indicate reasonable reliability in the testing techniques employed. Breeding for varieties with a definite tendency to cook white may be accomplished in the light of the behavior of the varieties considered in this investigation. If the 8 or 9 clonal selections are omitted, it will be noted that from a small sample of about 13 varieties selected at random 3 were found which had a tendency to cook white, 5 appeared to be intermediate in their behavior, and 5 had a tendency to cook dark. Two of the three varieties which exhibited white-cooking tendencies are closely related. The white-cooking Chippewa variety is one of the parents of the white-cooking Sebago variety. The inheritance of a physicochemical system, possibly the tyrosinase system, responsible for differences in the formation of dark melanin-like pigments in cooked potato-tuber tissue is suggested by the parent-offspring relationships of the following varieties:

Parents	*Offspring*
Chippewa (white) × Katahdin (intermediate)	Sebago (white).
Triumph (white) × Katahdin (intermediate)	Pontiac (intermediate).
Green Mountain (dark) × Katahdin (intermediate)	Sequoia (dark[4]).

The one common parent in these three crosses, Katahdin, has been classified as an intermediate variety. Its cooking behavior over a 5-year period places it in a borderline position between the white-cooking and intermediate-cooking classes. The white and intermediate varieties developed from Katahdin crosses indicate that this parent may carry genes for white-cooking quality. More definite genetic evidence for this assumption is furnished by the behavior of an F_1 population resulting from a cross between Katahdin and the dark-cooking Hindenburg variety recorded in table 2. Out of a population of 136 F_1 individuals, 58 produced less pigment than Katahdin, 45 reacted the same as Katahdin, while 18 behaved like the dark-cooking Hindenburg parent and 15 produced more pigment than the dark-cooking parental variety. A similar F_1 distribution was obtained from a cross between Sebago and Hindenburg. The progenies from both crosses exhibited transgressive inheritance with large representations of F_1 individuals on the white-cooking end of the blackening scale. These results show that white tuber flesh after cooking is dominant or incompletely dominant to gray tuber flesh after cooking, and that the genetic factorial interactions may be comparatively simple since the parental types were easily recovered in small populations of F_1 individuals. Segregation in the F_1 generation also shows that the varieties used in these crosses are heterozygous for genes controlling gray pigment formation in cooked potato-tuber tissue.

---

[4] The Sequoia rating is based on only 1 year's results at 9 locations. Its behavior suggests a borderline position between the intermediate-cooking and dark-cooking classes.

ENVIRONMENTAL INFLUENCES

Seasonal variations in growing and harvesting conditions certainly caused wide fluctuations in the incidence of blackening after cooking throughout the period of this study. This is clearly shown by the average blackening indices recorded in table 3 for nine varieties grown at nine widely separated locations. Almost a complete range of the blackening scale is represented with the high blackening index of 85 recorded for Oneida in 1937 and the low blackening index of 1 recorded for Walworth in 1941.

Climatic factors exerted a much greater influence on the incidence of blackening than soil types. Potatoes grown on the different soil types listed in table 3 all had comparatively high average blackening indices in 1937 and low blackening indices in 1941. In general, the potatoes grown on muck soils showed somewhat less blackening than those grown on upland mineral soils. Soil fertility levels, as indicated by potato yields, appeared to play a minor role in the development of potato blackening.

Air temperature appeared to be one of the most important environmental factors involved in after-cooking blackening. Low air temperatures during the months of June and September and high air temperatures during the month of August appeared to be associated with the development of blackening in this series of field experiments. These findings agree in part with the early field observation of Tottingham, Nagy, and Ross (*11*), who reported serious blackening difficulties in crops produced under high temperatures, and the conflicting field observation of Smith, Nash, and Dittman (*9*), who recently reported increased blackening of tubers which matured under low temperatures. Considerable progress might be made in an understanding of the nature of potato blackening after cooking by studying the relations between temperature and the occurrence of the blackening defect under controlled environmental conditions.

It is probable that some of the differences in amounts of blackening observed between varieties was due to environmental rather than genetic influences. Varieties of different maturities were planted at the same time at each location. Frequently the early-maturing varieties ripened during the hot month of August while the late-maturing varieties ripened during the latter part of September when the weather was usually much cooler. Judging from the observations made by Smith et al. (*9*), and partially substantiated in this study, it might be expected that the early-maturing varieties would tend to cook white while the late-maturing varieties would tend to cook dark. However, the varietal comparisons presented in table 1 show that this is not the case. Among the 6 early-maturing varieties, Triumph has a tendency to cook white, Red Warba and Earlaine are intermediate, while Irish

Cobbler, Mesaba, and White Blossom Cobbler have a tendency to cook dark. Of the 2 midseason varieties, Chippewa has a tendency to cook white and 100 Day Cobbler has a tendency to cook dark. The remaining 11 late-maturing varieties are distributed similarly to the early-maturing varieties with respect to cooking tendencies (table 7).

TABLE 7.—*Distribution of the 19 early, midseason, and late varieties of potatoes listed in table 1 according to their tendency to cook white, intermediate, or dark*

Variety group	Tendency to cook—		
	White	Intermediate	Gray
Early	1	2	3
Midseason	1	0	1
Late	1	3	7

In connection with these results the high positive correlation between mean air temperature during the month of August and the incidence of blackening may be important. The delay in harvesting the early-maturing varieties until late in the fall when the late-maturing varieties ripened may also have a bearing on the occurrence of blackening, since it is not known how or when the blackening mechanism operates. The conditioning of potatoes in storage had a marked effect on blackening. Freshly harvested samples which were held at room temperature for a few weeks prior to boiling frequently exhibited much less blackening than did comparable lots held in cold storage at 42° F. for a period of 2 months or more. These results substantiate the findings of Smith, et al. (*9*), who reported that high temperatures in storage for 3 to 4 days prevented blackening.

SUMMARY

Consistent differences in amounts of blackening after boiling were observed among 23 potato varieties and strains grown over a period of 5 years at 9 widely separated locations in Wisconsin. The 2 whitest-cooking varieties, Triumph and Chippewa, exhibited only one-half as much blackening as the 2 dark-cooking varieties, Rural New Yorker and Russet Rural. Eight named varieties developed through clonal selection behaved similarly to their parental varieties.

The white-cooking tendencies shown by the closely related Chippewa, Katahdin, and Sebago varieties suggest that these varieties carry genetic factors for white tuber flesh after boiling. The behavior of F_1 progenies derived from Katahdin and Sebago crossed with the dark-cooking Hindenburg variety supports this assumption. White tuber flesh after cooking was dominant or incompletely dominant to gray tuber flesh after cooking. Parental types were readily recovered among 267 F_1 individuals, indicating simple factorial interactions for after-cooking blackening. Segregation in the F_1 generation demonstrated heterozygosis in the parental stocks.

This study indicates that the dark-cooking character is inherited. By the breeding of potato varieties showing white tuber flesh after boiling this factor may be partially controlled.

Climatic factors exerted a profound influence on the formation of substances in potato tubers which turn dark after boiling, but the influence of soil type and soil fertility on the blackening process was limited. The conditioning of potatoes in storage had a marked effect on blackening.

LITERATURE CITED

(1) ASHBY, S. F.
1905-1906. A CONTRIBUTION TO THE STUDY OF FACTORS AFFECTING THE QUALITY AND COMPOSITION OF POTATOES. Jour. Agri. Sci. [England] 1: [347]-357.

(2) CLAGETT, C. O., and TOTTINGHAM, W. E.
1941. THE REDUCING-SUBSTANCE AND PHENOLIC-COMPOUND CONTENT OF THE POTATO TUBER IN RELATION TO DISCOLORATION AFTER COOKING. Jour. Agr. Res. 62: 349-358.

(3) HOTCHKISS, A., WOOD, M., and FINDLEN, P.
1940. COOKING-QUALITY PREFERENCES FOR POTATOES. Amer. Potato Jour. 17: [253]-261.

(4) MERKENSCHLAGER, F.
1929. ÜBER DAS SCHWARZWERDEN DER KARTOFFELKNOLLEN. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst 9: 20-21.

(5) NASH, L. B.
1941. POTATO QUALITY IV. RELATION OF VARIETY AND ENVIRONMENTAL CONDITION TO PARTIAL COMPOSITION AND COOKING QUALITY. Amer. Potato Jour. 18: [91]-99.

(6) ——— and SMITH, O.
1939. POTATO QUALITY II. RELATION OF MINERAL NUTRITION AND ALTERATIONS IN LIGHT INTENSITY TO COOKING QUALITY. Amer. Soc. Hort. Sci. Proc. 37: 861-865.

(7) PARKER, W. H.
1932. "QUALITY" IN POTATOES. Chem. and Indus. 51: 94-95.

(8) ROSS, A. F., and TOTTINGHAM, W. E.
1938. PROTEOLYTIC ACTIVITY IN RELATION TO THE BLACKENING OF POTATOES AFTER COOKING. Jour. Agr. Res. 57: 433-441, illus.

(9) SMITH, O., NASH, L. B., and DITTMAN, A. L.
1942. POTATO QUALITY VI. RELATION OF TEMPERATURE AND OTHER FACTORS TO BLACKENING OF BOILED POTATOES. Amer. Potato Jour. 19: 229-254.

(10) SPANGLER, R. L.
1940. RETAIL TRADE PRACTICES AND PREFERENCES FOR LATE-CROP POTATOES IN CHICAGO AND SUBURBS, AND QUALITY ANALYSES OF POTATOES OFFERED FOR SALE TO CONSUMERS, 1939-40. U. S. Agr. Marketing Serv., 66 pp. [Processed.]

(11) TOTTINGHAM, W. E., NAGY, R., and ROSS, A. F.
1936. THE PROBLEM OF CAUSES OF BLACKENING IN COOKED POTATOES. Amer. Potato Jour. 13: [297]-309, illus.

CHEMICAL COMPOSITION OF SOME AMERICAN WILD FEEDSTUFFS[1]

By THOMAS R. KING, formerly *collaborator*, and HAROLD E. MCCLURE, *assistant chemist, Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

In recent years much interest has been manifested in the conservation of wildlife. As a result, some work has been done to ascertain which wild plants will produce the largest and most nutritious supply of feed for wildlife, especially during the winter months, and to encourage the propagation of those feeds that hold the most promise. This work may be of value in the feeding of domestic animals, including poultry, when there is a shortage of farm crops. Although feeding tests are necessary in order to obtain the actual nutritive value of wild feedstuffs for domestic animals, data on their proximate composition will serve as a guide in indicating their probable nutritive value. A review of the literature shows that there is little information on the composition of wild feedstuffs.

Winton and Winton (*13*)[3] have compiled data on a few wild feedstuffs, but in many cases these data are not comprehensive enough to be of much value. Wehmer (*12*) made a fairly comprehensive compilation of wild feedstuffs found in Germany. Morrison (*9*) included, in his well-known tables of the composition of cultivated plants, the results of some analyses of a few wild feedstuffs. Recently Wainio and Forbes (*11*) published data on the proximate composition of 35 wild fruits and nuts. Bailey (*2, pp. 417–418*) reported the analyses of 62 native grains, seeds, and berries. A number of studies have been made of the chemical composition of wild plants and their seeds for the purpose of obtaining data on their pharmacological properties, but these data are, in most cases, of little or no value from the standpoint of animal nutrition.

The study reported here was undertaken for the purpose of obtaining more information on the composition of wild feedstuffs. The data should be useful in the fields of soil conservation, wildlife conservation, and animal nutrition. This work was conducted at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., by the Bureau of Animal Industry, the Bureau of Biological Survey (formerly a bureau of the United States Department of Agriculture but now a part of the Fish and Wildlife Service of the United States Department of the Interior), and the Virginia Polytechnic Institute.

---

[1] Received for publication April 23, 1943.

[2] The authors are indebted to Harry W. Titus, of the Bureau of Animal Industry, to Ralph B. Nestler and W. W. Bailey, who were employees of the Bureau of Animal Industry when this work was done, and to A. L. Nelson, of the Fish and Wildlife Service, Department of the Interior, for their assistance, advice, and helpful criticism.

[3] Italic numbers in parentheses refer to Literature Cited, p. 46.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 1
July 1, 1944
Key No. A-217

SOURCE OF SAMPLES AND METHODS USED

The samples of wild feedstuffs were obtained from several sources. Most of them were collected by the Bureau of Biological Survey during the fall of 1939 in various national forests, nurseries of the Soil Conservation Service, and forests of the Tennessee Valley Authority. Others were collected by the senior author during the fall and winter of 1939 and the spring of 1940. Some were purchased on the open market. Four were collected by Philip Barske of Ann Arbor, Mich., during the fall of 1940. The time of collection shown in table 1 is as accurate as it was possible to obtain from the records of the collectors. Additional information concerning the collection of the samples is recorded in table 1. The samples were classified as follows: (1) Legume seeds, (2) grass seeds, (3) miscellaneous seeds, (4) mast, (5) fruits, and (6) tubers.

In all but a few cases the samples, as received, were ground in a Wiley mill to pass through a 1-mm. sieve. The fleshy fruits, because of their perishable nature, were collected in the vicinity of the Beltsville Research Center and were dried as indicated in table 1 before grinding. The methods of the Association of Official Agricultural Chemists (*1*) were followed in the determination of moisture, crude protein, true protein, crude fiber, ether extract, total ash, calcium, and phosphorus.

ANALYTICAL RESULTS

The results of the chemical analyses were calculated to the moisture-free basis to make them comparable and are recorded in table 2. The moisture content of the samples at the time the analyses were made is also given in this table.

TABLE 1.—*Sample number, name, source, time of collection, and part analyzed of the products examined*

LEGUME SEEDS

Sample No.	Common name	Scientific name [1]	Source	Time of collection	Part analyzed [2]
	Pulse family:	Leguminosae:			
1	Bean, trailing wild	*Strophostyles helvola* (L.) Britton	Chapel Hill, N. C	Fall 1939	Seed without pod.
2	do	do	do	do	Sample 1 germinated.
3	do	do	Mayes County, Okla	1937	Seed without pod.
4	do	*Strophostyles* sp	Fredericksburg, Va	August 1939	Do.
5	Beggarweed, Florida	*Desmodium tortuosum* (Sw.) DC	Open market		Seed without hull.
6	Butterfly-pea	*Centrosema virginianum* (L.) Benth	Fredericksburg, Va	October 1939	Seed without pod.
7	Clover, crimson	*Trifolium incarnatum* L	do		Seed without hull.
8	Clover, hop	*Trifolium agrarium* L	Spartanburg, S. C	April 1940	Do.
9	Clover, rabbitfoot	*Trifolium arvense* L	Beltsville, Md	July 1939	Seed with hull.
10	Clover, tick	*Desmodium bracteosum* (Michx.) DC	Harrisburg, Ill	Fall 1939	Do.
11	Lespedeza	*Lespedeza cyrtobotrya* Miq	Chatham, Va	November 1939	Seed without hull.
12	do	*Lespedeza juncea* Pers	Thorsby, Ala		Do.
13	do	*Lespedeza virginica* (L.) Britton	Beltsville, Md	November 1939	Seed with hull.
14	Lespedeza, common	*Lespedeza striata* (Thunb.) H. and A. var. Kobe	Alabama		Do.
15	Lespedeza, Korean	*Lespedeza stipulacea* Maxim	Open market		Seed without hull.
16	do	do	Alabama		Do.
17	do	do	do		Sample 16 germinated.
18	Lespedeza, sericea	*Lespedeza cuneata* G. Don	Open market		Seed without hull.
19	do	do	Beltsville, Md	September 1939	Do.
20	do	do	do	do	Seed with hull.
21	Lespedeza, shrub	*Lespedeza bicolor* Turcz	Chatham, Va	November 1939	Seed without hull.
22	Locust, black	*Robinia pseudoacacia* L	Frederick, Md	September 1939	Seed without pod.
23	do	do	Zanesville, Ohio	Fall 1939	Do.
24	do	do	do	do	Sample 23 germinated.
25	Partridgepea	*Cassia chamaecrista* L	Richmond, Va	March 1939	Seed without pod.
26	do	do	Mayes County, Okla	1937	Do.
27	do	*Cassia* sp	Illinois	Fall 1939	Do.
28	Plant, wild sensitive	*Cassia nictitans* L	Thorsby, Ala		Do.
29	Sweetclover, white	*Melilotus alba* Desr	Open market		Seed without hull.
30	Trefoil, birdsfoot	*Lotus corniculatus* L	do		Do.
31	Vetch, common	*Vicia angustifolia* L	do		Do.

See footnotes at end of table.

TABLE 1.—*Sample number, name, source, time of collection, and part analyzed of the products examined*—Continued

GRASS SEEDS

Sample No.	Common name	Scientific name [1]	Source	Time of collection	Part analyzed [2]
	Grass family:				
	Miscellaneous grasses:	Gramineae:			
32	Crabgrass	*Digitaria sanguinalis* (L.) Scop	Decatur, Ala	October 1939	Seed with hull.
33	do	do	Elsberry, Mo		Do.
34	Foxtail grass	*Setaria lutescens* (Weigel) F. T. Hubb	Fredericksburg, Va	August 1939	Do.
35	Redtop	*Agrostis alba* L	Open market		Do.
36	Ryegrass	*Lolium sp*	do		Do.
	Panic grasses:				
37	Fall panic grass	*Panicum dichotomiflorum* Michx	Cache, Okla	September–October 1939	Do.
38	Panic grass	*Panicum agrostoides* Spreng	Decatur, Ala	October 1939	Do.
39	do	*Panicum anceps* Michx	Forest City, Ark	October–November 1939	Do.
40	do	*Panicum fasciculatum* Swartz	Texas		Do.
41	do	*Panicum texanum* Buckl	do		Do.
42	Witchgrass	*Panicum capillare* L	Hamburg, La	Fall 1939	Do.
43	Switchgrass	*Panicum virgatum* L	San Antonio, Tex		Do.
	Paspalum grasses:				
44	Bull grass	*Paspalum boscianum* Flügge	Thomasville, Ga	September–October 1939	Do.
45	Dallis grass	*Paspalum dilatatum* Poir	Shreveport, La	October 1939	Do.
46	Paspalum grass	*Paspalum floridanum* Michx	Hattiesburg, Miss	Fall 1939	Do.
47	do	*Paspalum laeve* Michx	Fredericksburg, Va	September 1939	Do.

MISCELLANEOUS SEEDS

Sample No.	Common name	Scientific name [1]	Source	Time of collection	Part analyzed [2]
	Buckwheat family:	Polygonaceae:			
48	Sorrel, sheep	*Rumex acetosella* L	Beltsville, Md	June 1940	Seed with hull.
49	Smartweed	*Polygonum setaceum* Baldw	Decatur, Ala	October 1939	Do.
50	do	*Polygonum* sp	Greenbelt, Md	December 1939	Seed without hull.
	Composite family:	Compositae:			
51	Ragweed	*Ambrosia aptera* DC	Texas		Seed.
52	do	*Ambrosia artemisiifolia* L	Ithaca, N. Y		Do.
53	Madder family: Buttonweed, narrow-leaved.	Rubiaceae: *Diodia teres* Walt	Open market		Do.
54	Touch-me-not family: Jewelweed	Balsaminaceae: *Impatiens pallida* Nutt	Beverly, W. Va	September–October 1939	Do.

MAST

	Beech family:	Fagaceae:			
55	**Oak, bear**	*Quercus ilicifolia* Wang	Wrightstown, N. J	Fall 1939	Acorn without cup.
56	**Oak, blackjack**	*Quercus marilandica* Muench	Hattiesburg, Miss	do	Do.
57	do	do	do	do	Do.
58	do	do	Springfield, Mo	do	Do.
59	**Oak, bluejack**	*Quercus cinerea* Michx	Hattiesburg, Miss	do	Do.
60	**Oak, chestnut**	*Quercus prinus* L	Arlington, Va	September 1939	Do.
61	do	do	Elk Range, Va	Fall 1939	Do.
62	**Oak, Havard's**	*Quercus havardi* Rydb	Beckham County, Okla	do	Do.
63	**Oak, live**	*Quercus virginiana* Mill	Hattiesburg, Miss	do	Do.
64	**Oak, Nuttall's**	*Quercus nuttallii* Palmer	St. Charles, Ark	do	Do.
65	**Oak, pin**	*Quercus palustris* Muench	Harrisburg, Ill	do	Do.
66	**Oak, post**	*Quercus stellata* Wang	Blacksburg, Va	do	Do.
67	do	do	Cache, Okla	September-October 1939	Do.
68	**Oak, Spanish**	*Quercus falcata* Michx	Hattiesburg, Miss	Fall 1939	Do.
69	**Oak, water**	*Quercus nigra* L	Decatur, Ala	October 1939	Do.
70	do	do	St. Charles, Ark	Fall 1939	Do.
71	do	do	Hattiesburg, Miss	do	Do.
72	do	do	do	do	Sample 71 germinated.
73	**Oak, white**	*Quercus alba* L	Elk Range, Va	Fall 1939	Acorn without cup.
74	**Oak, willow**	*Quercus phellos* L	Decatur, Ala	October 1939	Do.
75	do	do	Fredericksburg, Va	September 1939	Do.
76	do	do	Monticello, Ark	November 1939	Do.
77	do	do	St. Charles, Ark	Fall 1939	Do.
78	do	do	do	do	Sample 77, shell only.[3]
79	do	do	do	do	Sample 77, kernel only.[4]
80	Birch family: Hazelnut	Betulaceae: *Corylus* sp			Nut without involucre.
	Pine family:	Pinaceae:			
81	**Pine loblolly**	*Pinus taeda* L	Open market		Seed without wing.
82	**Pine, longleaf**	*Pinus palustris* Mill	Hattiesburg, Miss	October 1939	Do.
83	**Pine, ponderosa**	*Pinus ponderosa* Laws	Elsberry, Mo		Do.
84	**Pine, shortleaf**	*Pinus echinata* Mill	Springfield, Mo	Fall 1939	Do.
85	Witch-hazel family: Sweetgum	Hamamelidaceae: *Liquidambar styraciflua* L	Tennessee	1937	Do.

See footnotes at end of table.

Table 1.—*Sample number, name, source, time of collection, and part analyzed of the products examined*—Continued

FRUITS

Sample No.	Common name	Scientific name [1]	Source	Time of collection	Part analyzed [2]
86	Barberry family: Barberry, Japanese	Berberidaceae: *Berberis thunbergii* DC	Beltsville, Md	April 1940	Whole fruit.
87	Buckthorn family: Plum, hog	Rhamnaceae: *Colubrina texensis* Gray	San Antonio, Tex		Do.
	Cashew family:	Anacardiaceae:			
88	Skunkbush	*Rhus canadensis* var. *trilobata* (Nutt.) Gray.	Sayre, Okla	Fall 1939	Seed without pulp.
89	Sumac, dwarf	*Rhus copallina* L	Hattiesburg, Miss	do	Whole fruit.
90	do	do	Decatur, Ala	October 1939	Pulp without seed.
91	do	do	Lancaster, Pa	November 1939	Seed without pulp.
92	Sumac, smooth	*Rhus glabra* L	Fredericksburg, Va	September 1939	Whole fruit.
93	do	do	Wenatchee, Wash	Fall 1939	Do.
94	do	do	Washtenaw County, Mich.	November-December 1940.	Do.
95	do	do	Okanogan, Wash	October 1939	Pulp without seed.
96	Sumac, staghorn	*Rhus typhina* L	Lancaster, Pa	November 1939	Seed without pulp.
	Ebony family:	Ebenaceae:			
97	Persimmon, common	*Diospyros virginiana* L	Beltsville, Md	January 1940	Pulp without seed [5]
98	do	do	do	do	Seed without pulp.[5]
	Honeysuckle family:	Caprifoliaceae:			
99	Coralberry	*Symphoricarpos orbiculatus* Moench	Greenbelt, Md	December 1939	Whole fruit.[6]
100	Honeysuckle	*Lonicera morrowii* Gray	Beltsville, Md	June 1940	Do.[5]
101	Honeysuckle, Japanese	*Lonicera japonica* Thunb	Berwyn, Md	November 1939	Do.[6]
102	Lily family: Saw brier	Liliaceae: *Smilax glauca* Walt	Greenbelt, Md	December 1939	Do.[6]
	Nettle family:	Urticaceae:			
103	Hackberry	*Celtis pallida* Torr	San Antonio, Tex		Do.
104	Mulberry	*Morus alba* L	Beltsville, Md	June 1940	Do.[5]
105	do	*Morus* sp	Virginia	June 1939	Do.[5]
106	Oleaster family: Russian-olive	Elaeagnaceae: *Elaeagnus angustifolia* L	Albuquerque, N. Mex	November 1938	Do.
107	Olive family: Privet	Oleaceae: *Ligustrum obtusifolium* var. *regelianum* (Koehne) Rehd.	Beltsville, Md	April 1940	Do.
	Rose family:	Rosaceae:			
108	Cherry, wild black	*Prunus serotina* Ehrh	Fredericksburg, Va	September 1939	Fruit with stone.[6]
109	Dewberry, wild	*Rubus* sp	Beltsville, Md	July 1940	Whole fruit.[5]
110	Plum, beach	*Prunus maritima* Marsh	Sandy Hook, N. J	September 1939	Stones, cleaned.[5]
111	Rose, Japanese	*Rosa multiflora* Thunb	Open market		Whole fruit.
112	Rose, rambling	*Rosa rugosa* Thunb	Beltsville, Md	March 1940	Do.
113	Rose, swamp	*Rosa palustris* Marsh	Blacksburg, Va	November 1939	Do.
114	do	do	do	April 1940	Do.
115	Strawberry, wild	*Fragaria* sp	Greenbelt, Md	June 1940	Fruit with calyx.[5]

	Sweetgale family:	Myricaceae:			
116	Bayberry	*Myrica pensylvanica* Lois	Wrightstown, N. J	Fall 1939	Whole fruit.
117	Waxmyrtle	*Myrica cerifera* L	Thorsby, Ala		Do.
118	do	do	Virginia Beach, Va	Fall 1939	Do.
119	Vine family: Grape, Frost	Vitaceae: *Vitis vulpina* L	Washtenaw County, Mich.	October-November 1940	Do.[6]
		TUBERS			
120	Sedge family: Chufa	Cyperaceae: *Cyperus esculentus* L	Open market		Tubers.

---

[1] Authority for nomenclature is the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[2] Unless otherwise indicated the part analyzed was air-dried.

[3] 30.08 percent of whole acorn.

[4] 69.92 percent of whole acorn.

[5] Dried at 100° C.

[6] Dried at room temperature.

TABLE 2.—*Percentage analyses of the products examined*

LEGUME SEEDS

Sample No.	Common name	Moisture content when analyzed	Proximate chemical composition on moisture-free basis								
			Crude protein [1]	True protein [2]	Nonprotein nitrogen compounds [3]	Crude fiber	Nitrogen-free extract	Fat, or ether extract	Ash	Calcium	Phosphorus
	Pulse family:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Bean, trailing wild	9.08	32.92	31.08	1.39	10.98	51.92	0.58	4.05	0.10	0.60
2	do	6.81	33.16	29.68	2.62	11.21	51.54	.60	4.35		
3	do	10.43	31.75	28.89	2.15	11.50	52.50	.64	4.32	.13	.60
4	do	9.39	32.81	30.33	1.87	13.12	49.16	.81	4.71	.14	.54
5	Beggarweed, Florida	8.47	36.55	32.16	3.30	13.71	37.80	9.52	3.51	.15	.67
6	Butterfly-pea	9.44	26.48	21.71	3.59	10.44	58.50	2.78	2.98	.24	.38
7	Clover, crimson	7.46	41.65	36.05	4.21	11.43	42.19	1.86	4.26	.13	.79
8	Clover, hop	7.08	42.19	33.87	6.25	9.13	37.10	10.20	3.45	.08	.69
9	Clover, rabbitfoot	7.16	20.69	19.30	1.04	26.12	46.39	2.77	4.38	1.23	.37
10	Clover, tick	6.11	22.41	18.13	3.22	28.85	34.84	5.24	9.72	.76	.44
11	Lespedeza	7.22	35.59	31.85	2.81	12.60	35.44	12.33	4.97	.17	.59
12	do	8.67	42.72	39.35	2.53	11.17	36.89	6.64	3.42	.14	.49
13	do	7.58	33.19	29.12	3.06	16.71	42.01	4.54	4.56	.61	.54
14	Lespedeza, common	7.66	43.94	40.91	2.29	12.03	33.20	6.95	4.62	.29	.83
15	Lespedeza, Korean	9.02	43.60	41.41	1.65	9.80	33.10	8.75	5.29	.40	.82
16	do	7.16	40.73	38.84	1.42	11.02	35.74	8.38	4.60	.38	.87
17	do	5.68	43.72	35.54	6.16	11.79	32.97	8.25	5.29		
18	Lespedeza, sericea	8.73	38.02	34.16	2.90	13.88	41.39	4.02	3.65	.14	.50
19	do	7.05	38.36	34.39	2.98	13.33	40.13	4.96	4.21	.15	.59
20	do	7.23	32.34	29.27	2.31	16.71	42.72	4.65	4.34	.58	.50
21	Lespedeza, shrub	7.75	38.44	35.60	2.14	13.07	33.97	11.11	4.11	.17	.54
22	Locust, black	7.42	46.65	34.96	8.79	16.33	23.64	11.97	4.31	.25	.90
23	do	6.33	46.83	35.85	8.26	15.60	25.15	10.46	4.68	.23	.79
24	do	5.40	50.34	35.32	11.30	17.05	21.29	10.44	4.60		
25	Partridgepea	9.21	36.16	33.74	1.82	7.99	45.49	6.63	4.33	.59	.46
26	do	8.92	39.89	35.89	3.01	8.65	42.84	5.39	4.22	.63	.49
27	do	8.11	40.06	35.54	3.40	8.81	45.00	3.40	3.85	.62	.54
28	Plant, wild sensitive	10.24	40.92	35.46	4.10	7.68	45.03	3.03	4.70	.51	.55
29	Sweetclover, white	7.82	40.56	36.32	3.19	12.30	39.79	4.52	3.88	.30	.59
30	Trefoil, birdsfoot	8.54	37.92	32.52	4.06	14.13	39.69	5.39	4.21	.32	.56
31	Vetch, common	8.73	34.71	29.89	3.63	7.11	55.20	.48	3.69	.20	.54

GRASS SEEDS											
	Grass family:										
	Miscellaneous grasses:										
32	Crabgrass	12.08	13.55	12.73	0.61	15.45	61.59	3.30	6.32	0.13	0.36
33	do	9.29	12.82	12.34	.36	14.85	63.53	3.62	5.30	.12	.41
34	Foxtail grass	7.16	14.28	13.91	.28	67.57	8.63	.42	9.19	.10	.25
35	Redtop	6.83	20.26	19.52	.56	10.34	55.57	7.03	6.98	.31	.58
36	Ryegrass	8.86	8.99	8.38	.45	10.56	74.20	2.05	4.36	.23	.35
	Panic grasses:										
37	Fall panic grass	7.68	15.21	13.96	.93	19.89	51.70	4.13	9.39	.13	.34
38	Panic grass	11.36	12.94	12.44	.37	23.23	54.02	.88	9.06	.20	.33
39	do	5.65	11.79	11.56	.17	29.05	51.48	2.08	5.66	.16	.23
40	do	6.96	14.37	12.76	1.21	29.58	43.49	2.98	9.98	.30	.21
41	do	6.61	17.36	15.50	1.39	23.20	50.60	4.39	4.92	.12	.26
42	Witchgrass	9.12	12.80	12.04	.57	19.67	54.08	3.09	10.55	.34	.39
43	Switchgrass	5.72	15.00	14.26	.56	14.63	59.29	5.24	6.02	.12	.39
	Paspalum grasses:										
44	Bull grass	9.57	7.70	7.55	.11	17.95	65.75	3.82	4.82	.14	.38
45	Dallis grass	5.49	9.00	7.83	.88	33.57	44.72	5.02	7.98	.15	.37
46	Paspalum grass	8.98	9.16	8.64	.40	28.44	55.99	1.81	4.72	.09	.14
47	do	6.69	6.19	5.32	.66	43.28	44.83	1.03	4.88	.08	.13
MISCELLANEOUS SEEDS											
	Buckwheat family:										
48	Sorrel, sheep	10.71	8.20	7.63	0.43	18.74	62.97	3.29	6.94	0.29	0.26
49	Smartweed	6.80	11.72	10.23	1.13	23.74	58.44	1.80	4.66	.48	.38
50	do	9.21	10.78	10.28	.39	18.37	65.12	3.84	2.00	.11	.29
	Composite family:										
51	Ragweed	3.83	21.11	19.54	1.19	27.57	24.57	22.10	5.03	.71	.58
52	do	7.08	24.50	23.54	.73	34.10	24.53	13.47	3.63	.36	.56
53	Madder family: Buttonweed, narrow-leaved	5.55	12.45	11.29	.88	44.90	28.29	3.65	10.99	.61	.19
54	Touch-me-not family: Jewelweed	6.01	28.23	27.75	.36	2.95	19.59	44.55	4.80	.47	.97
MAST											
	Beech family:										
55	Oak, bear	9.62	6.10	5.94	0.12	18.27	54.61	19.41	1.65	0.19	0.10
56	Oak, blackjack	14.65	6.29	6.28	.01	20.94	60.11	10.66	2.00	.37	.09
57	do	7.77	5.48	5.07	.30	21.79	52.63	18.18	2.03	.40	.11
58	do	8.21	5.75	5.49	.20	21.79	53.03	17.73	1.76	.36	.09
59	Oak, bluejack	15.71	5.92	5.92	.09	12.52	67.06	12.66	1.84	.33	.09
60	Oak, chestnut	6.89	6.42	6.06	.28	15.22	72.93	3.34	2.17	.14	.12
61	do	7.37	3.58	3.56	.02	20.32	60.35	13.97	1.78	.13	.08

See footnotes at end of table.

TABLE 2.—*Percentage analyses of the products examined*—Continued

MAST—Continued

Sample No.	Common name	Moisture content when analyzed	Proximate chemical composition on moisture-free basis								
			Crude protein [1]	True protein [2]	Nonprotein nitrogen compounds [3]	Crude fiber	Nitrogen-free extract	Fat, or ether extract	Ash	Calcium	Phosphorus
	Beech family—Continued.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
62	Oak, Havard's	9.01	7.73	6.28	1.09	12.22	71.42	6.65	2.34	0.14	0.11
63	Oak, live	10.17	5.22	4.52	.52	16.71	67.95	8.59	1.71	.18	.08
64	Oak, Nuttall's	5.65	3.83	3.57	.19	13.24	66.65	14.99	1.36	.17	.05
65	Oak, pin	8.81	7.16	6.37	.59	15.58	58.37	17.82	1.27	.13	.08
66	Oak, post	7.70	5.15	4.89	.20	19.46	66.74	5.74	2.97	.40	.11
67	do	8.24	4.83	4.59	.19	19.61	65.23	7.60	2.78	.38	.10
68	Oak, Spanish	10.64	4.20	4.16	.02	20.60	57.73	15.57	1.92	.43	.08
69	Oak, water	7.11	4.27	4.20	.05	17.60	54.17	22.83	1.15	.29	.08
70	do	7.66	3.97	3.89	.06	18.87	54.16	21.79	1.23	.21	.06
71	do	7.53	3.67	3.64	.02	18.99	54.82	20.86	1.67	.30	.04
72	do	5.10	4.59	4.50	.07	19.60	50.49	23.73	1.61	------	------
73	Oak, white	9.67	6.73	5.85	.66	17.38	67.25	5.67	3.19	.19	.14
74	Oak, willow	11.10	4.24	4.08	.12	19.36	54.74	19.61	2.09	.30	.02
75	do	13.37	5.08	5.06	.02	18.82	55.87	18.52	1.71	.30	.07
76	do	9.32	4.61	4.34	.20	18.28	52.84	22.99	1.35	.28	.08
77	do	11.33	4.93	4.92	.01	18.63	52.79	21.86	1.79	.29	.08
78	do	7.55	2.47	2.41	.04	54.57	41.07	0.51	1.40	.69	.02
79	do	5.63	5.81	5.80	.01	2.51	60.42	29.52	1.74	.10	.12
80	Birch family: Hazelnut	5.97	6.72	6.61	.09	51.98	19.32	20.53	1.47	.33	.10
	Pine family:										
81	Pine, loblolly	7.36	15.88	15.63	.18	56.45	16.98	6.74	4.02	.04	.35
82	Pine, longleaf	6.88	33.85	33.17	.50	23.93	8.59	28.87	4.94	.08	.73
83	Pine, ponderosa	7.13	21.28	21.10	.13	37.77	14.22	21.97	4.81	.06	.61
84	Pine, shortleaf	7.41	28.63	28.10	.40	32.95	9.88	22.10	6.57	.08	.76
85	Witch-hazel family: Sweetgum	4.91	26.01	25.32	.52	17.95	33.62	13.86	8.73	1.22	.57

FRUITS

Sample No.	Common name	Moisture content when analyzed	Crude protein [1]	True protein [2]	Nonprotein nitrogen compounds [3]	Crude fiber	Nitrogen-free extract	Fat, or ether extract	Ash	Calcium	Phosphorus
86	Barberry family: Barberry, Japanese	12.54	15.25	12.81	1.84	10.78	62.32	8.24	4.01	0.33	0.33
87	Buckthorn family: Plum, hog	4.05	19.25	17.94	.99	31.21	30.40	17.42	2.04	.34	.22
	Cashew family:										
88	Skunkbush	5.71	15.81	14.19	1.22	25.97	43.94	12.81	1.87	.08	.31
89	Sumac, dwarf	5.65	8.21	7.80	.31	28.54	42.88	17.17	3.30	.16	.16
90	do	5.47	4.88	4.68	.15	20.66	57.65	10.83	6.03	.85	.10

91	....do	7.06	13.37	12.74	.47	29.74	41.92	12.91	2.22	.14	.37
92	Sumac, smooth	8.63	5.28	5.10	.13	35.11	46.41	10.05	3.20	.47	.23
93	....do	4.59	4.97	4.94	.02	31.61	46.58	13.53	3.32	.41	.25
94	....do	5.33	5.22	4.90	.24	28.78	45.83	17.21	3.04	.48	.24
95	....do	6.17	3.02	2.78	.18	21.91	50.03	15.59	9.51	.94	.10
96	Sumac, staghorn	4.88	10.75	10.18	.43	28.64	46.27	11.94	2.54	.18	.48
	Ebony family:										
97	Persimmon, common	4.51	3.27	2.87	.30	5.29	87.44	.89	3.21	.12	.08
98	....do	5.89	9.57	8.68	.67	23.61	63.00	2.02	2.02	.13	.20
	Honeysuckle family:										
99	Coralberry	6.98	8.50	7.72	.59	21.40	59.78	6.27	4.24	.40	.22
100	Honeysuckle	3.50	7.97	7.17	.60	9.09	75.44	3.89	3.81	.31	.24
101	Honeysuckle, Japanese	10.56	10.42	8.81	1.21	6.65	69.37	7.86	6.10	.66	.31
102	Lily family: Saw brier	7.58	10.24	8.12	1.59	18.59	60.77	7.53	3.40	.25	.16
	Nettle family:										
103	Hackberry	4.47	8.23	7.42	.61	8.95	45.81	4.75	32.46	14.56	.19
104	Mulberry	3.08	8.96	7.91	.78	9.98	67.86	8.07	5.40	.67	.33
105	....do	3.10	5.46	3.99	1.10	5.02	80.04	2.56	7.29	.60	.20
106	Oleaster family: Russian-olive	9.91	9.45	7.54	1.43	20.29	64.08	4.53	2.13	.22	.04
107	Olive family: Privet	11.66	12.76	12.04	.53	13.70	56.25	13.38	4.10	.36	.29
	Rose family:										
108	Cherry, wild black	6.71	13.94	12.88	.79	32.66	43.00	6.97	3.70	.42	.20
109	Dewberry, wild	3.47	5.79	5.47	.24	21.50	64.39	4.89	3.51	.33	.19
110	Plum, beach	5.50	6.50	6.14	.28	57.93	23.55	11.26	.84	.15	.11
111	Rose, Japanese	5.56	10.04	9.49	.41	23.14	56.56	6.07	4.33	.90	.33
112	Rose, rambling	9.46	10.98	9.94	.78	40.09	40.11	6.19	2.89	.86	.19
113	Rose, swamp	5.74	10.76	9.90	.65	30.87	47.06	7.02	4.50	.89	.20
114	....do	6.20	10.16	9.69	.35	31.97	45.94	7.47	4.58	.95	.26
115	Strawberry, wild	2.46	7.59	7.13	.35	20.54	58.25	6.93	6.80	.65	.29
	Sweetgale family:										
116	Bayberry	6.19	7.08	7.02	.04	41.52	24.88	24.78	1.78	.13	.03
117	Waxmyrtle	6.80	5.56	5.47	.06	42.10	29.04	21.81	1.52	.26	.04
118	....do	7.38	5.88	5.74	.11	40.30	21.23	31.00	1.62	.11	.11
119	Vine family: Grape, Frost	7.90	9.82	8.51	.98	22.21	54.36	9.52	4.42	.52	.26
	TUBERS										
120	Sedge family: Chufa	9.86	4.96	2.60	1.78	8.96	55.41	29.54	1.71	.03	.61

[1] 6.25 × total nitrogen.

[2] 6.25 × albuminoid nitrogen.

[3] 4.7 × difference between total and albuminoid nitrogen.

The ranges in composition of the groups into which the samples were classified are shown in table 3. Several of the feedstuffs whose composition was not representative of their groups were omitted from the table. Since wide ranges were found even within the same botanical family groups, it was in many instances difficult to classify the wild feedstuffs into groups having distinctly definite characteristics.

TABLE 3.—*Ranges of composition of the groups*

Group name	Moisture content when analyzed	Range of chemical composition on moisture-free basis								
		Crude protein [1]	True protein [2]	Nonprotein nitrogen compounds[3]	Crude fiber	Nitrogen-free extract	Fat, or ether extract	Ash	Calcium	Phosphorous
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Legumes (seed)	5-10	21-50	18-41	1-11	7-29	21-58	0.5-12	3-10	0.1-1.2	0.4-0.9
Grasses (seed) [4]	6-12	6-20	5-20	.1-1.4	10-43	43-74	.9- 7	4-10	.1- .3	.1- .6
Miscellaneous (seed) [5]	4-11	8-24	8-24	.4-1.2	18-34	25-65	1.8-22	2- 7	.1- .7	.2- .6
Mast (seed) [6]	6- 7	16-34	16-33	.1- .5	24-56	9-17	7-29	4- 7	.0- .1	.4- .8
Mast (nuts) [7]	5-16	4- 8	4- 6	0-1.1	12-22	50-73	3-24	1- 3	.1- .4	0- .1
Fruits [8]	3-13	3-19	3-18	0-1.8	5-42	21-87	.9-31	2- 9	.1-1.0	0- .5
Tubers	10	5	3	2	9	55	30	2	0.03	.6

[1] 6.25×total nitrogen.
[2] 6.25×albuminoid nitrogen.
[3] 4.7×difference between total and albuminoid nitrogen.
[4] Excluding foxtail grass (sample 34).
[5] Excluding buttonweed (sample 53) and jewelweed (sample 54).
[6] Excluding sweetgum (sample 85).
[7] Excluding willow oak (shell only and kernel only (samples 78 and 79), and hazelnut (sample 80).
[8] Excluding hackberry (sample 103) and beach plum (sample 110).

The legume seeds had a high crude protein content, but in many cases a considerable portion of the nitrogen was in the form of nonprotein nitrogen compounds. They also contained considerable nitrogen-free extract (carbohydrates), moderate quantities of fat and crude fiber, and more calcium and phosphorus than the domestic cereal grains.

The grass seeds in general were comparable to wheat and corn but contained slightly more protein, considerably more crude fiber, and less nitrogen-free extract. Foxtail grass seed was a marked exception in this group, since it had a very high content of crude fiber and a very low content of nitrogen-free extract.

Among the miscellaneous seeds a considerable range in composition was found, but this was to be expected since four families were represented. Jewelweed seeds were notably high in fat, protein, and phosphorus, but contained little crude fiber.

The mast group may conveniently be divided into seeds and nuts. The seeds were high in protein and crude fiber and low in nitrogen-free extract, whereas the nuts were low in protein and phosphorus and high in nitrogen-free extract. The quantity of fat found in both seeds and nuts varied considerably. Hazelnuts were very high in crude fiber, probably because of their thick shell, and low in nitrogen-free extract. Sweetgum seeds contained much more calcium than the other mast samples. One sample of acorns was separated into shell (sample 78) and kernel (sample 79) and the two parts were analyzed separately. The results indicate that the nutritive value of the acorn depends on whether the shell is included in the diet of the animal.

The fruits varied greatly in composition. This group was characterized in the main by a high content of nitrogen-free extract. The fruit of the sweetgale family, however, had only a moderate content of nitrogen-free extract and a rather high content of crude fiber and fat. Separate analyses of the seed (samples 91 and 96) and pulp (samples 90 and 95) of the sumac fruit indicate that the seed contains more protein and phosphorus but much less ash and calcium than the pulp. The fruit of the sumac probably has little nutritive value since Errington (*4*) found that it failed to maintain the live weight of captive bobwhite quail when fed as the sole source of feed. This observation was confirmed by Nestler and Bailey,[4] who showed that captive bobwhite quail under very favorable conditions of shelter and temperature lost weight when the only feedstuff supplied to them was sumac fruit, pulp, or seed. The fruit of the hackberry was unusual in that the ash and calcium content was exceptionally high.

Chufa, the only member of the tuber group analyzed, had a high content of fat and nitrogen-free extract, but contained little protein.

DISCUSSION

Certain factors that influence the chemical composition of feedstuffs should be considered. The type of soil in which a plant is grown undoubtedly has some effect on the chemical composition of the fruit and seed. This subject has been adequately treated by Browne (*3*) and many others, but Hart, Guilbert, and Goss (*6*), in discussing range forages, stated that the difference in composition between species is more marked than the variation within the species when grown on different areas. The stage of development of the plant and the conditions of exposure to the weather to which it has been subjected influence to a great extent the chemical composition of the fruit and seed. Guilbert, Mead, and Jackson (*5*) reported that seeds collected relatively late in the season may have some of their nutritive value leached out. This confirms the earlier report of Le Clerc and Breazeale (*8*), who stated that when plants dry and wilt the inorganic constituents exude to the surface, from which they may be easily washed by rain.

It is necessary to make feeding tests before attempting to arrive at definite conclusions regarding the value of wild feedstuffs in the diet of wild and domestic animals and birds. Many of these feedstuffs, such as sumac, may be found to be of doubtful value. Others may yield excellent results with some animals and poor results with others. The beneficial effect of one nutritive constituent may be overbalanced by the harmful effect of another. For example, King and Titus (*7*) found in experiments with growing chicks that acorns of the willow oak are a rich source of vitamin A, comparable with alfalfa-leaf meal, but Olsen (*10*) showed that when hens were fed diets containing 25 percent of meat or hulls of the acorns of either the white or black oak, they produced eggs having yolks of an undesirable, greenish color.

---

[4] Unpublished data of R. B. Nestler and W. W. Bailey, of the Patuxent Research Refuge, Bowie, Md.

SUMMARY

Chemical analyses were made of 120 samples of American wild feedstuffs. The samples included legume seeds, grass seeds, miscellaneous seeds, mast, fruits, and tubers.

Legume seeds have good nutritive value as indicated by high protein and nitrogen-free extract content. Grass seeds and fruits are of lower nutritive value and supply mainly nitrogen-free extract, although some of the fruits contain considerable fat. The seeds of the mast group are concentrated feedstuffs having, in general, a high protein and a low nitrogen-free extract content, whereas the nuts of this group have a low protein and a high nitrogen-free extract content. The quantity of fat found in both seeds and nuts varies considerably.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1935. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus., Washington, D. C.

(2) BAILEY, E. M.
1942. COMMERCIAL FEEDING STUFFS. REPORT OF INSPECTION, 1941. Conn. Agr. Expt. Sta. Bul. 459, 422 pp.

(3) BROWNE, C. A.
1938. SOME RELATIONSHIPS OF SOIL TO PLANT AND ANIMAL NUTRITION—THE MAJOR ELEMENTS. U. S. Dept. Agr. Yearbook (Soils and Men) 1938: 777–806, illus.

(4) ERRINGTON, P. L.
1937. EMERGENCY VALUES OF SOME WINTER PHEASANT FOODS. Wis. Acad. Sci., Arts, Letters, Trans. 30: 57–68.

(5) GUILBERT, H. R., MEAD, S. W., and JACKSON, H. C.
1931. THE EFFECT OF LEACHING ON THE NUTRITIVE VALUE OF FORAGE PLANTS. Hilgardia 6: 13–26.

(6) HART, G. H., GUILBERT, H. R., and GOSS, H.
1932. SEASONAL CHANGES IN THE CHEMICAL COMPOSITION OF RANGE FORAGE AND THEIR RELATION TO NUTRITION OF ANIMALS. Calif. Agr. Expt. Sta. Bul. 543, 62 pp., illus.

(7) KING, T. R., and TITUS, H. W.
1943. ACORNS OF THE WILLOW OAK, QUERCUS PHELLOS, A SOURCE OF VITAMIN A ACTIVITY. Poultry Sci. 22: 56–60.

(8) LE CLERC, J. A., and BREAZEALE, J. F.
1909. PLANT FOOD REMOVED FROM GROWING PLANTS BY RAIN OR DEW. U. S. Dept. Agr. Yearbook 1908: 389–402.

(9) MORRISON, F. B.
1936. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 20, unabridged, 1050 pp., illus. Ithaca, N. Y.

(10) OLSEN, M. W.
1942. ACORNS ONE CAUSE OF OLIVE-COLORED YOLKS. Poultry Sci. 21: 497–499.

(11) WAINIO, W. W., and FORBES, E. B.
1941. THE CHEMICAL COMPOSITION OF FOREST FRUITS AND NUTS FROM PENNSYLVANIA. Jour. Agr. Res. 62: 627–635.

(12) WEHMER, C.
1929–1931. DIE PFLANZENSTOFFE . . . Ed. 2, 2 v. Jena.

(13) WINTON, A. L., and WINTON, K. B.
1932. THE STRUCTURE AND COMPOSITION OF FOODS. Vol. 1, illus. New York and London.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

Vol. 69 WASHINGTON, D. C., JULY 15, 1944 No. 2

COMPARISON OF SHOOT APEX AND LEAF DEVELOPMENT AND STRUCTURE IN DIPLOID AND TETRAPLOID MAIZE[1]

By L. F. RANDOLPH, *cytologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture,* and *professor of botany, New York State College of Agriculture;* ERNST C. ABBE, *associate professor of botany, University of Minnesota;* and JOHN EINSET, *assistant in plant breeding, New York State College of Agriculture*

INTRODUCTION

It is well known that autopolyploids, originating by direct doubling of the chromosome complement, often are morphologically different from the parental forms. The term "gigas," which has been associated with the autotetraploid state since the early work on *Oenothera*, carries with it a series of connotations concerning the manner in which the form of the tetraploid organism differs from that of its diploid relatives. But as East (*4*)[2] and others have emphasized, not all autopolyploids are true gigas types. Numerous qualitative observations have been made on the morphology of experimentally induced autotetraploids, and the investigations in this field were comprehensively reviewed by Müntzing (*6*) in 1936. In the past few years additional qualitative observations have been made, especially on the mature state, yet relatively few precise quantitative morphological studies of autotetraploids have been made.

It is becoming increasingly apparent that the effects of direct chromosome doubling are extremely diverse, especially when attention is focused on specific or individual characteristics. These effects may differ not only in different species but also in different genetic entities within the species. We are here dealing with inherited form differences which, ostensibly at least, cannot be attributed to gene differences of the ordinary sort. Obviously, the change from the diploid to the tetraploid state involves a doubling of the number of genes, but there is presumably no change in the kinds or relative proportions of the genes. It is extremely important to know why it is that different genotypes respond differently to chromosome doubling. In certain instances it may be due to cumulative versus noncumulative gene action, as suggested by Randolph and Hand (*10*) in connection with analyses of the carotenoid content of diploid and tetraploid

---

[1] Received for publication February 27, 1943. Cooperative investigations of the New York State College of Agriculture; the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; and the University of Minnesota, aided by a grant from the Committee on Radiations of the National Research Council. Assistance at the University of Minnesota was provided through the National Youth Administration, which made available the services of Don. L. Jacobs and Max Schuster.

[2] Italic numbers in parentheses refer to Literature Cited, p. 76.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 2
July 15, 1944
Key No. G-1307

maize; but little is known about the genetics of morphological differences induced by chromosome doubling.

With respect to these morphological differences, it is of primary importance to determine just what their essential nature is, both in the developmental history of the plant and at maturity. In attempting critical morphological comparisons of comparable diploids and tetraploids, the quantitative approach is of basic significance since it permits more accurate characterization of the effect of doubling the number rather than modifying the quality of the genes. Accordingly, quantitative studies of both developmental and mature manifestations of autotetraploidy in maize were undertaken with due regard to possible genetic implications.

The leaf was selected as perhaps the most suitable organ of the plant for such comparative morphological analyses, as it has a relatively simple form whose external dimensions and internal cellular constituents can be measured with reasonable accuracy at all stages of development. In diploid maize the increased width of successively younger leaves, from leaf 6 through leaf 12, is definitely correlated with an increase in the size of the shoot apex (*2*). With this evidence at hand, it seemed especially desirable to investigate the size relations of cells, tissues, and organs in the shoot apex and the leaf during ontogeny and at maturity in comparable strains of diploid and tetraploid maize.

MATERIAL AND METHODS

The diploid and tetraploid strains used in the ontogenetic studies had a common origin. Tetraploidy was induced by heat treatment (*7*) in an F_1 hybrid between inbred lines of Webbers Dent and Illinois A, pollinated by an inbred line of Luces Favorite. The resultant tetraploid plants, several in number, were then mass-pollinated for 2 generations to provide adequate material for the investigation. Diploid sister plants of the original induced tetraploids were similarly mass-pollinated for 2 generations to produce a diploid strain as nearly comparable to the tetraploid as possible. These were the strains utilized by Randolph and Hand (*10*) in a study of the relation between carotenoid content and the number of genes per cell in diploid and tetraploid maize. To insure the selection of comparable diploid and tetraploid material from these stocks, a considerable number of ear-row progenies were grown from the diploid and tetraploid strains, from which were selected 4 tetraploid and 4 diploid ears whose progenies were representative of the range of variation in the 2 strains. From each ear a minimum of 120 kernels were planted in 5 replications and grown during the summer of 1938 in a soil-filled bench in the greenhouses of the Botany Department, Cornell University. Similar plantings were also made in the experimental gardens of the Department of Plant Breeding, Cornell University, to provide material for a comparison of mature-leaf dimensions in field-grown plants. Additional stocks utilized in the comparisons of mature-leaf dimensions will be described later.

Details concerning collections, dissection techniques, fixing, sectioning, and staining applicable to the following developmental study have been published elsewhere (*2*). The histological studies of the mature leaf described in the second part of this paper involved the use of either frozen microtome sections or hand sections of fresh material. For these studies hand sections proved eminently satisfactory, not only because they could be prepared expeditiously, but also because a higher degree of accuracy in quantitative histological studies results from a minimum of departure from the living state.

The tenth leaf blade, i. e., the leaf blade located at the tenth node above the coleoptile, was selected as representative of the approximately 16 leaves ordinarily produced by maize varieties of this locality. It was evident, from the relative growth curves obtained in the previous study of leaf development in diploid maize (*2*), that the leaves produced during the period of maximum vegetative activity (leaves 6 through 12) are similar in general growth pattern; but because of the consistent differences between successive leaves, it seemed desirable to select a leaf at one particular position for the present study. Therefore, the tenth leaf (counting the first leaf above the coleoptile as leaf 1) was selected for detailed analysis. This is the leaf that ordinarily subtends the functional ear shoot of the mature plant.

Since each leaf originates as a primordium that develops as a ridge about the base of the shoot apex, an understanding of the developmental relations of the leaf requires a knowledge of the organization of the shoot apex itself.

SHOOT APEX DURING PLASTOCHRON 10

The shoot apex of maize in the seedling stage is an elongate paraboloid of revolution, from the base of which arise the primordia of the leaves in acropetal succession (fig. 1). In the mature kernel the coleoptile and five seedling leaves already are partly formed, and in the developing seedling and young plant additional primordia develop successively at intervals of 2 to 3 days under optimum growing conditions. The primordium of the tenth leaf is normally being formed in seedlings about 8 to 10 days after planting. The time interval during which the leaf primordium is differentiating from the shoot apex is designated by Askenasy's term "plastochron." When the tenth leaf primordium is differentiating, the shoot apex is said to be in the tenth plastochron. The shoot apex during the tenth plastochron formed the basis for the following observations.

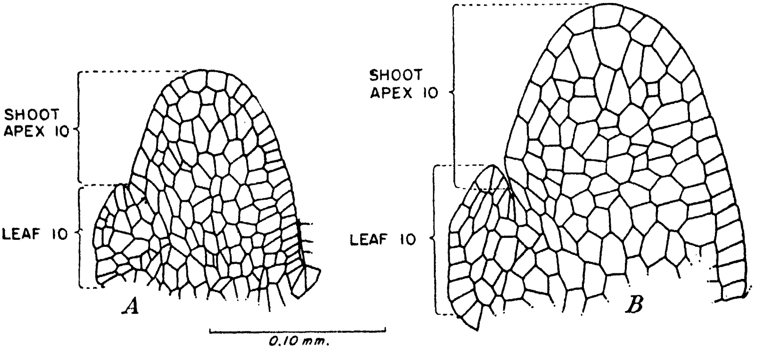


FIGURE 1.—Shoot apex and primordium of leaf 10 in median longitudinal section, showing relative dimensions of cell and organ in (*A*) diploid and (*B*) tetraploid maize. Camera lucida drawings of representative shoot apices.

It was readily possible in the seedling stage to dissect out separately the shoot apex, the leaf primordia, and the immature leaves at various stages of development, by means of the technique described by Abbe, Randolph, and Einset (*2*). The entire shoot apex and the young leaves were measured directly to determine their gross dimensions. Thereafter, a certain number of these same shoot apices were embedded in paraffin and sectioned to provide material for detailed analyses of their cellular structure.

The absolute size of the diploid shoot apex during the tenth plastochron differed markedly from that of the tetraploid. The basal diameter of the diploid averaged 130μ as contrasted with an average of 154μ for the tetraploid. In length the diploid averaged 73μ and the tetraploid 94μ (table 1). Thus the tetraploid shoot apex was very significantly larger than the diploid shoot apex.

TABLE 1.—*Size relations of diploid and tetraploid shoot apices during plastochron 10*

Item	Data on shoot apices			N[1]	Method of obtaining measurements
	Diploid	Tetraploid	Ratio ($4n/2n$) on a linear basis		
1. Basal diameter .... μ	[2] 130.0±[3] 3.0	154.0±[3] 3.0	[4] 1.19*	25	Direct measurement.
2. Length .... μ	73 ± 3.0	94 ± 3.0	1.29*	15	Do.
3. Basal circumference μ	408	484	1.19	...	Item 1$\times\pi$.
4. Area in median section μ^2	6,300	9,600	1.24($\sqrt{1.53}$)		$\frac{2}{3}\, d\, h$.
5. Volume .... μ^3	485,000	875,000	1.22($\sqrt[3]{1.81}$)	...	$\frac{1}{2}\pi r^2 h$.
6. Cells in median longitudinal section. No	60.8± 4.4	56.8± 2.7	.93	16	Direct observation.
7. Average cell area .... μ^2	104	169	1.28($\sqrt{1.63}$)		Item 4/item 6.
8. Cells in basal diameter. No	10.1± .4	9.9± .4	.98†	16	Direct observation.
9. Average cell diameter μ	12.9	15.6	1.21		Item 1/item 8.
10. Average cell volume μ^3	1,342	2,636	1.25($\sqrt[3]{1.96}$)		Item 7$\times$item 9.
11. Nuclear diameter μ	7.4± .08	9.3± .08	1.26*	200	Direct measurement.
12. Nuclear volume .... μ^3	212	421	1.26($\sqrt[3]{1.99}$)	...	$\frac{4}{3}\pi r^3$.

[1] N=total number of samples.

[2] The slight discrepancy between this value and the corresponding one in Abbe, Randolph, and Einset (*2*) is to be attributed chiefly to a difference in the population sampled.

[3] Standard error.

[4] Student's t was used to determine the significance of the difference between tetraploid and diploid. An asterisk (*) indicates that the odds were greater than 19:1 and that the values are therefore significantly different; a dagger (†) indicates that the values are not significantly different.

The absolute dimensions of the diploid and tetraploid shoot apices were used to compute certain other size relationships. From the basal diameter may be computed the basal circumference of the shoot apex (table 1, item 3), which is also a measure of the lateral extent of the leaf primordium at the time of its inception, since the young leaf primordium in maize originates as a circumferential structure at the base of the shoot apex. From a comparison of the computed basal circumferences of the diploid and tetraploid, it was apparent that the basal circumference of the tetraploid was 1.19 times that of the diploid. This is an important relationship, since it determines the relative widths of the leaf primordia at the time of their origin. As will be shown later, there is a close correlation between the width or circumferential extent of the leaf primordium in the diploid and the tetraploid and the ratio (1.16) between the width of the tetraploid leaf and that of the diploid, for any given length during their subsequent development.

The absolute dimensions of the diploid and tetraploid shoot apices were used also in computing their relative volumes. The shape of the shoot apex is that of a paraboloid of revolution. By utilizing the formula for determining the volume of such a structure (*2*), as shown in table 1, item 5, it was found that the volume of the tetraploid was 1.81 times that of the diploid, a value which is equivalent on a linear basis to 1.22 ($\sqrt[3]{1.81}$) times. The shoot apex is in itself an important morphological entity during an extended period in the ontogeny of the plant. Since various organs of the mature plant are derived from the shoot apex, the fact that polyploidy directly influences its volume relations is of primary importance in any consideration of the developmental relations of these organs.

Since the tetraploid shoot apex was larger than that of the diploid (fig. 1 and table 1), the question arose as to the relation of cell number and cell size to organ size. The cells were counted in the basal diameter of median longitudinal sections of diploid and tetraploid shoot apices. In the diploid the average number of cells was 10.1, and in the tetraploid 9.9 (table 1, item 8). There was no statistically significant difference between these two values. By dividing the basal diameter of the shoot apices by the number of cells in the basal diameter it was determined that the average diameter of the diploid cells was 12.9μ and the average diameter of the tetraploid cells was 15.6μ (table 1, item 9), the average diameter of the tetraploid cells being 1.21 times that of the diploid.

The average area of individual cells in the diploid and tetraploid shoot apices was computed by dividing the average median longitudinal area of the shoot apex by the average number of cells in the median longitudinal plane of section. The resultant values, computed on the assumption that the median plane of section had the form of a paraboloid, are given in table 1. The average sectional area of the tetraploid cells was 169μ^2 and that of the diploid cells was 104μ^2. The average number of cells in the median plane of section was 56.8 for the tetraploid and 60.8 for the diploid, there being no statistically significant difference between these two numbers. However, the average area of the tetraploid shoot apex in median longitudinal section was approximately 50 percent greater than that of the diploid, the actual values being 9,600μ^2 and 6,300μ^2, respectively. The average area of the individual tetraploid cells in the median longitudinal plane of section was 1.63 times, or on a linear basis, 1.28 ($\sqrt{1.63}$) times that of the individual diploid cells (table 1, item 7). This ratio is in reasonably good agreement with that obtained for the comparison of cell diameters.

The average cell volume in the diploid and in the tetraploid shoot apex, obtained by multiplying cell area by cell diameter (table 1, item 10), was found to be in the tetraploid nearly twice (1.96 times) that in the diploid, which corresponds to a linear relationship of 1.25 ($\sqrt[3]{1.96}$) times.

It was anticipated that chromosome doubling would affect nuclear size as well as cell size. Nuclear volume, computed from direct measurements of nuclear diameter (table 1, item 12), was found to be twice (actually 1.99 times) as great in the tetraploid as in the diploid. This was to be expected, since the tetraploid nuclei contained twice as many chromosomes as the diploid.

From these computations of nuclear and cell relations it was apparent that in the shoot apex chromosome doubling resulted in a doubling of nuclear and cell volume without any change in cell number or cell pattern, and the volume of the tetraploid shoot apex was also essentially twice as great as that of the diploid. But this simple relationship did not prevail in the mature leaf blade, either in the form or pattern of the leaf as a whole or in the cells and tissues of which it was composed, as will be shown later.

DEVELOPMENT OF THE TENTH LEAF BLADE

In the initial stages of development the tenth leaf of the tetraploid was greater in width than that of the diploid. This was clearly due to the increased size of the tetraploid shoot apex. In maize the primordium of the leaf develops as a ridge that almost completely surrounds the base of the shoot apex, and since the circumference of the tetraploid shoot apex was greater than that of the diploid there was a corresponding increase in the width of the embryonic tetraploid leaf. In tracing the subsequent development of the leaf it was at once apparent that this initial advantage in width of the tetraploid was not maintained in terms of amount of growth in width per unit of time. The absolute and relative dimensions of the leaf blade at maturity were also indicative of altered developmental relationships.

RATE OF INCREASE IN WIDTH AND LENGTH

Since the relation of one dimension to another in development (see fig. 5) is a reflection of the rate at which each of the dimensions changes (figs. 2 and 3), it seemed desirable first to consider separately the rate of increase in length and in width of the diploid and tetraploid maize leaves.

The rates of increase in the length and width of the tenth leaf are represented in figure 2, *A* and *B*, respectively. In each figure the

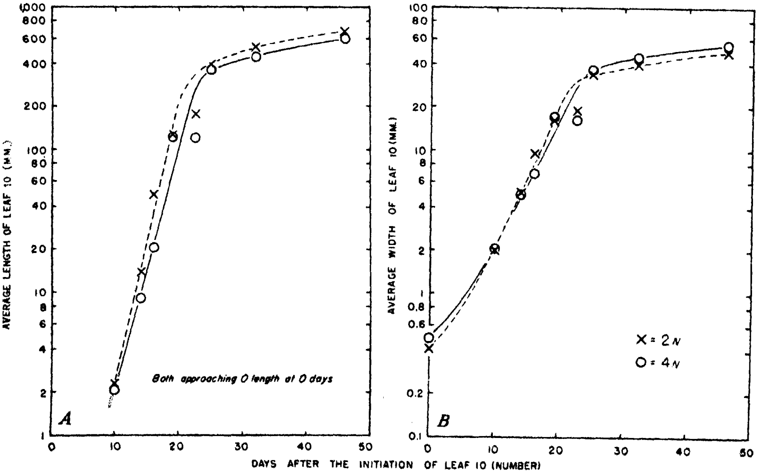


FIGURE 2.—Growth rate of leaf 10 in (*A*) length and (*B*) width in diploid and tetraploid maize. The dimension of the leaf is plotted logarithmically on the ordinate. Time is plotted arithmetically on the abscissa.

linear dimension (on the ordinate) is plotted logarithmically. The size of the sample taken on any one day averaged 20 leaves (varying from 16 to 24), the plants being chosen to include the greatest range in size for a particular day, except for the twenty-second and twenty-third days after the origin of the leaf initial when only smaller leaved plants were taken. The latter exception explains the deviation from the trend exhibited by the values for that collection (fig. 2, *A* and *B*).

The rates of increase in length (fig. 2, *A*) of the diploid and tetraploid leaf blades differed slightly, and the time at which they approached maturity was also somewhat different. In measuring leaf elongation during ontogeny both diploid and tetraploid tenth leaves may be considered to start at zero length. Subsequently the tetraploid elongated at a slightly lower rate than the diploid, so that its absolute length as development proceeded became a smaller and smaller proportion of the length of the diploid at the same harvests (table 2). But the diploid, after having grown more rapidly in length, also began to mature sooner (see "break" in curve for 2*n*, fig. 2, *A*), so that the tetraploid leaf, through a later inception of the maturation process, nearly reached the length of the diploid at maturity (fig. 2, *A*, and table 2). In other strains, studied only at maturity (table 3), it was found that the tetraploid may surpass the diploid in its mature leaf length. In the growth of the leaf blade in length there are, then, at least two important factors, which may well be independent variables (perhaps subject to separate genic control), one being the absolute growth rate and the other the threshold of maturation.

TABLE 2.—*Ratios of widths and of lengths of the tenth leaves of tetraploid and diploid maize plants at different stages of development* [1]

Dimension	Ratio (4*n*/2*n*) at indicated number of days from initiation of leaf								
	0	10	14	16	19	22-23	25	32	46
Length		0.89	0.65	0.43	0.96	0.68	0.93	0.85	0.90
Width	1.19	1.02	.97	.72	1.06	.86	1.08	1.11	1.14

[1] Based upon a total of 316 leaves of each type.

TABLE 3.—*Length and width of mature tenth leaves of diploid and tetraploid maize*

Strain	Stock		Where grown	Leaf length			Leaf width			
	Diploid	Tetraploid		Diploid	Tetraploid	Ratio (4*n*/2*n*)	Diploid	Tetraploid	Ratio (4*n*/2*n*)	Leaves measured
				Cm.	*Cm.*		*Cm.*	*Cm.*		*Number*
A	3760A	3759A	Greenhouse.	67.2±[1]2.5	60.2±2.3	0.90	4.8±0.2	5.5±0.2	1.15	26
A′	3760A	3759A	Field	73.8± 3.9	70.9±3.8	.96	4.7± .2	5.3± .2	1.13	22
B	41410	41409	do	82.4± 1.6	83.7±2.5	1.02	9.5± .3	11.5± .4	1.21	20
C	41437	41436	do	73.8± 1.3	81.7±2.2	1.11	9.5± .2	10.9± .3	1.15	20
D	41459	41457	do	82.6± 1.0	82.2±1.6	1.00	9.1± .3	11.0± .2	1.21	20
E	41449	41446	do	65.4± .9	66.9±1.0	1.02	9.3± .2	10.0± .4	1.08	20
Mean						1.00±.03			1.16±.02	

[1] Standard error.

The rates of increase in the width of the leaf (fig. 2, *B*) also differed in the diploid and the tetraploid. Basically the relationships between the curves are like those for length except that the starting points for the width comparisons differ, whereas the starting points for the length comparisons are the same. The tetraploid leaf blade increased in width more slowly than the diploid and entered the maturation phase somewhat later. The tetraploid leaf primordium was wider than the diploid, but this initial advantage was not maintained by the developing leaf blade. It remained wider for a relatively brief period. Then, because of its slower growth rate, its width approached that of the diploid and then fell below it, but ultimately surpassed it again because of the later onset of maturation (fig. 2, *B*, and table 2). At maturity, the width of the tetraploid leaf was 1.14 times that of the diploid, nearly equivalent to its initial width advantage (1.19 times); but in the intervening period it had fallen below and again surpassed the diploid. Thus, the initial size of the organ, growth rate, and maturation threshold are important factors influencing form determination.

The rates of increase in width and in length show a basic similarity in the relative behavior of the diploid and tetraploid leaf blades. In comparison with the diploid, the rate of increase of the tetraploid leaf blade is slower for both width and length, but this is partly compensated by the delay in the time of maturation.

INCREASE IN WIDTH AS RELATED TO INCREASE IN LENGTH

The marked similarity between the rates of growth in length and in width of the tetraploid (and also of the diploid) is clearly apparent not only when the single dimension is considered in relation to the time factor (fig. 2, *A* and *B*), but also when the dimensions are considered in relation to each other (fig. 3, *A* and *B*). The data are plotted arithmetically in figure 3, *A*, and logarithmically in figure 3, *B*, the latter having the advantage that the parallel nature of the curves makes visually evident the fact that, for any given length, the tetraploid leaf is wider than the diploid by a uniform percentage. It is thus obvious that while the tetraploid leaf blade grows more slowly than the diploid, width increases in proportion to length in the same way that it does in the diploid; for any given increase in length there was a proportional increase in width in both the diploid and the tetraploid. However, the tetraploid leaf started as a structure of greater lateral extent, while length increase started at essentially zero in each case. The tetraploid leaf blade had an initial advantage in width (1.19 times), which was maintained at about the same level (1.16 times) throughout development. It should be kept in mind that this is a comparison of one dimension with another, in contrast to the rate (dimension against time) comparisons described under the preceding heading. The growth in width relative to length was, then, similar in the diploid and the tetraploid, but for any given width of the diploid at a given length the tetraploid leaf at that same length was on the average 1.16 times as wide. This value was based on measurements of 316 leaves representing various developmental stages from a length of 1.5 mm. to maturity. The measurements were seriated on the basis of length, and the corresponding lengths and widths were averaged in groups of 10, beginning with the smallest. These averages were plotted arithmetically (fig. 3, *A*). By means of this arithmetic graph (in which the amount of distortion in the central and upper portions was less than in the logarithmic graph), the average widths of the diploid and tetraploid leaves were determined, by graphic interpolation at length intervals of 100 mm., in leaves ranging from

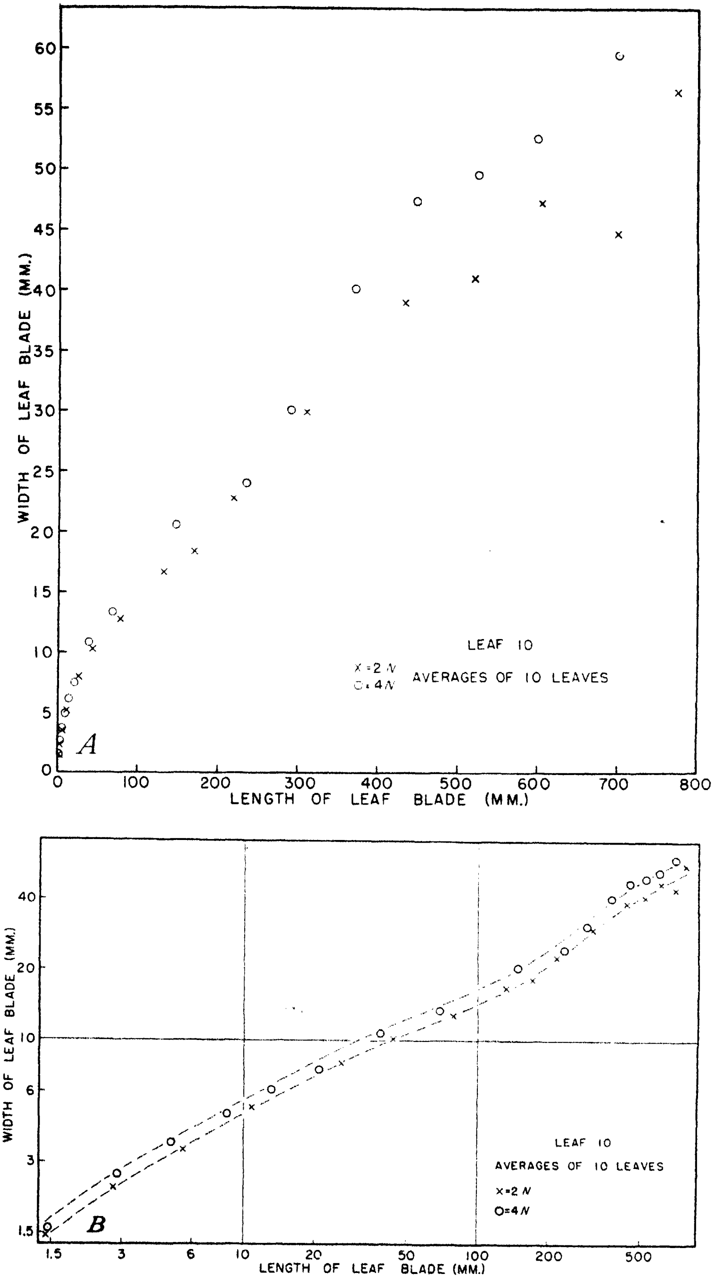


FIGURE 3.—Relation of leaf width to length during development in diploid and tetraploid maize, plotted (*A*) arithmetically and (*B*) logarithmically.

100 mm. to 700 mm. in length. The ratio of the width of the tetraploid leaf to that of the diploid for a given length was computed for each length class and was found to be on the average 1.16±0.03. There was relatively little departure from the average at any stage in development. It is undoubtedly significant that this value is essentially the same as the ratio (1.19) between the width of the tetraploid leaf initial and that of the diploid.

The results of the study of the developmental patterns of the diploid and tetraploid leaf blades may be summarized as follows. (1) For any unit of time, the diploid leaf blade increased more than the tetraploid in length and width and also started to mature sooner. At maturity the tetraploid approached the diploid very closely in length, although it was definitely shorter than the diploid during intermediate stages of development; and in width the tetraploid surpassed the diploid at maturity, having started at a greater width but fallen behind during its earlier development. (2) The slower rate of increase in width and length of the tetraploid is of such a nature that the relative increase in width for a given increase in length is very similar to that in the diploid. Thus, for any unit of length increase, there is a proportional increase in width in both the diploid and tetraploid, but the width of the tetraploid is 1.16 times that of the diploid, irrespective of stage of development, this being closely correlated with the initial advantage (1.19 times) in width of the tetraploid leaf primordium.

GROSS MORPHOLOGY OF THE MATURE TENTH LEAF BLADE

The external form, as well as the internal cellular organization, of the mature tenth leaf was compared in five different strains (A, B, C, D, and E, table 3) and in a sectorial chimera in which comparable diploid and tetraploid material was available. Strain A was the same strain as that used in the studies of the shoot apex and leaf development previously described, and both greenhouse-grown (A) and field-grown (A′) diploid and comparable tetraploid plants were examined. The tetraploid strains B, C, and D originated from a chromosome-doubled hybrid involving diploid inbred lines of Illinois A, Webbers Dent, and Luces Favorite, the three strains being segregated as separate lines after three generations of inbreeding and thereafter self-pollinated for an additional four generations. The tetraploid strain E originated from a chromosome-doubled hybrid of two morphologically similar lines of Spanish Flint, which was subsequently inbred for five generations. Diploid stocks of each of the four tetraploid strains B, C, D, and E were obtained from the selfed progeny of parthenogenetic diploids, which arose as spontaneous mutants in the tetraploid strains during the generation immediately preceding that in which the comparisons were made. (See Randolph and Fischer (*9*) for a discussion of parthenogenesis in tetraploid maize.) In addition to the studies of the tenth leaf in these five strains, the third leaf of a plant that was half diploid and half tetraploid and had appeared in a heat-treated genetic culture was also studied (fig. 4). One-half of each leaf of this plant was tetraploid and the other half diploid, the line of demarcation between the two being in the region of the midrib. This chimera furnished ideal material for a critical comparison of diploid and tetraploid leaf tissue, since the possibility of the existence of genetic or environmental differences in such material could be disregarded. The tenth leaf in the mature plants of strain A ordinarily subtended the functional ear shoot, and this criterion was utilized in selecting leaves for measurement from the mature plants of strains B, C, D, and E.

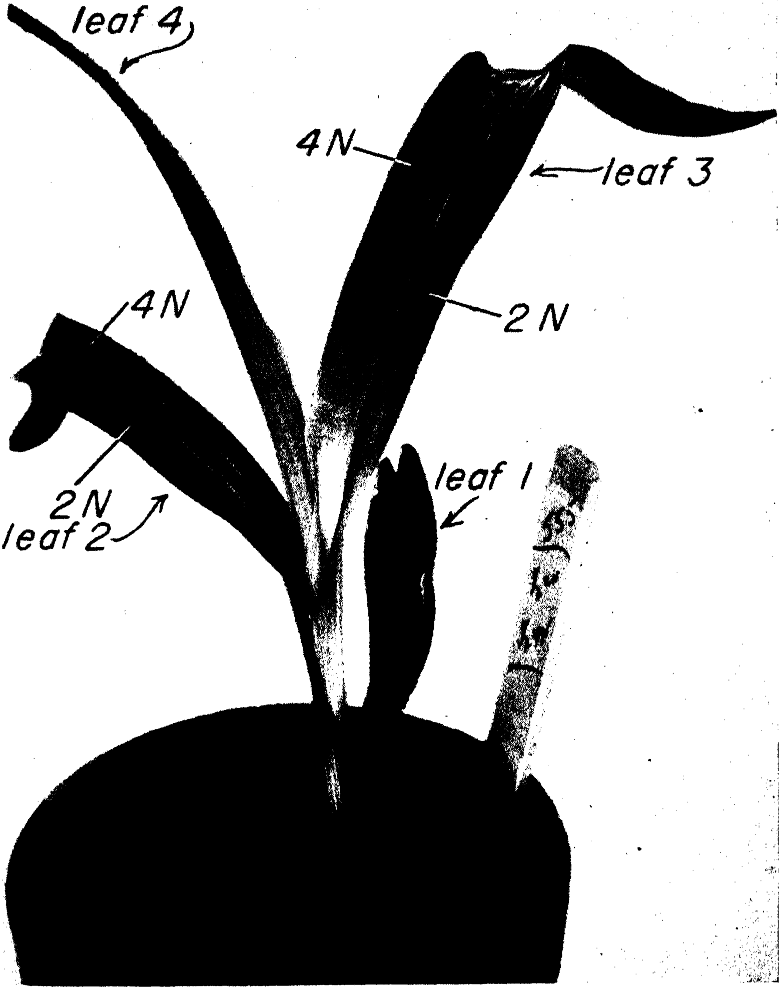


FIGURE 4.—A sectorial chimera in maize. The portion of each leaf labeled "4*n*" is tetraploid from the edge to the midrib and for the full length of the leaf, while the rest of each leaf (labeled "2*n*") is diploid.

The length and width measurements of the tenth leaf in strains A, A′, B, C, D, and E are summarized in table 3. The leaves of both the diploid and tetraploid plants of strain A were notably narrower for their length than those of the other strains. This might have been due partly to the fact that this strain was not planted until July 1, whereas the others were planted about May 15. The lengths of the tetraploid and diploid leaves were very similar in four of the five sets of comparisons made on full-grown plants; in the fifth comparison (strain C) the tetraploid leaf blades were on an average significantly longer than the diploid. However, the tetraploid leaves were shorter than the diploid leaves in the greenhouse material of strain A; in the field-grown plants of this strain the lengths were not significantly different. The combined average lengths of the diploid and tetraploid leaves for all six comparisons were exactly the same.

The tetraploid leaves were significantly wider than the diploid leaves in all of the strains, the average increase in width of the tetraploid leaves being 1.16 times that of the diploid leaves. Although the absolute widths of the leaves in the later planted field and greenhouse material of strain A were much less than those of the other four strains, which were planted at the earlier normal planting date, the tetraploid leaves exceeded the diploid in width to essentially the same degree in all of the strains. It is especially noteworthy that the tetraploid leaves were consistently wider than the diploid leaves in all of the comparisons that were made. Furthermore, in these five strains the mean width ratio of the tetraploid mature leaves to the diploid (1.16) coincided very closely with the average value obtained for the entire ontogenetic development of the leaf in strain A.[7] Because of the greater size of the shoot apex, the tetraploid leaf was wider than the diploid in the initial stages of development, and there was a similar consistent difference in the widths of the diploid and tetraploid leaves at maturity, although their lengths at maturity were essentially the same.

The thickness of the diploid and tetraploid leaf blades was measured in strains B, C, D, and E from either hand sections or frozen microtome sections of freshly harvested leaves, the measurements being taken in the region midway between the margin and the midrib of the leaf at a point approximately 15 cm. from the base of the leaf blade. The thickness of the diploid and tetraploid halves of the third leaf of the sectorial chimera previously mentioned was measured from permanent mounts, the leaf tissue having been fixed in alcohol, sectioned in paraffin, and stained with haematoxylin. All measurements were made with an ocular micrometer at a magnification of approximately 330, which permitted a very high degree of accuracy.

There was an unexpected uniformity in the absolute thickness of both the diploid and the tetraploid tenth leaf blades (table 4). In each of the four strains the average thickness of the diploid leaves was 0.26 mm. and the average thickness of the tetraploid leaves 0.35 mm. Thus the tetraploid tenth leaf was 1.35 times as thick as the comparable diploid leaf. This striking uniformity in leaf thickness in the different diploid and tetraploid strains is remarkable, in view of the differences in the average lengths and widths of the leaves in these same strains, and suggests that leaf thickness in maize is less subject to genetic variation than are the two other dimensions of the leaf.

The diploid and tetraploid halves of leaf 3 of the sectorial chimera (table 4) were somewhat thinner than the comparable regions of the tenth leaves of strains B, C, D, and E, and the tetraploid half was only 1.2 times as thick as the diploid half.

TABLE 4.—*Comparison of leaf thickness in mature diploid and tetraploid leaf blades*

Strain	Stock		Type of section	Leaf No.	Thickness of leaves at maturity						Ratio (*4n/2n*)
					Diploid			Tetraploid			
	Diploid	Tetraploid			Leaves measured	Measurements of thickness	Mean thickness and standard error	Leaves measured	Measurements of thickness	Mean thickness and standard error	
					Number	*Number*	*Millimeter*	*Number*	*Number*	*Millimeter*	
B	41409	41410	Hand	10	5	100	0.26±[1]0.10	5	100	0.35±[1]0.01	1.35
C	41437	41436	do	10	5	100	.26± .01	5	100	.35± .01	1.35
D	41459	41457	Frozen	10	10	545	.26± .01	10	335	.35± .01	1.35
E	41449	41446	Hand	10	5	100	.26± .01	5	100	.35± .01	1.35
Average							.26			.35	1.35
Chimera	A70	A70	Paraffin	3	1	49	.13± .01	1	65	.15±.003	1.15

[1] Standard error of individual means.

The length, width, and thickness of the tenth leaf blade having been determined, it was possible to compute the approximate volume of the diploid and tetraploid leaves in the various strains. The surface of the tenth leaf blade at maturity closely approximated the form of an isosceles triangle, and for the purpose of volume comparisons it was assumed that the leaf has a uniform thickness throughout, although in this study sufficient measurements were not taken in different regions of the leaf to verify this assumption.

The difference in the volumetric relations of the mature diploid and tetraploid leaves was not so great as was the difference in the volume of the shoot apices. The volume of the tetraploid shoot apex was, on an average, 1.80 times that of the diploid shoot apex, whereas the volume of the mature tenth leaf in the tetraploid plants was 1.54 times that of the comparable leaf in the diploid plants. These differences in volume relations were presumably related to the differences in the response of the various dimensions of the mature leaf blade to chromosome doubling. On a linear basis the average width of the tetraploid leaf was 1.16 times that of the diploid and its thickness was 1.33 times that of the diploid, whereas their lengths were the same. Expressed on a linear basis the volume of the tetraploid shoot apex was 1.22 times the volume of the diploid shoot apex. Chromosome doubling resulted in an essentially symmetrical size increase of the shoot apex and an asymmetrical increase in the various dimensions of the mature leaf blade. The manner in which the quantitative size relations in the diploid and tetraploid leaves were related to the cellular organization and internal structure of the leaf remains to be discussed.

HISTOLOGY OF THE MATURE TENTH LEAF

The tenth leaf blade of maize has a definite cell pattern when viewed in cross section (fig. 5). The upper and lower epidermal tissues are each one cell layer in thickness. Their component cells are elongated parallel to the long axis of the leaf blade and tend to be slightly wider (in dimension parallel to the leaf surface and perpendicular to the

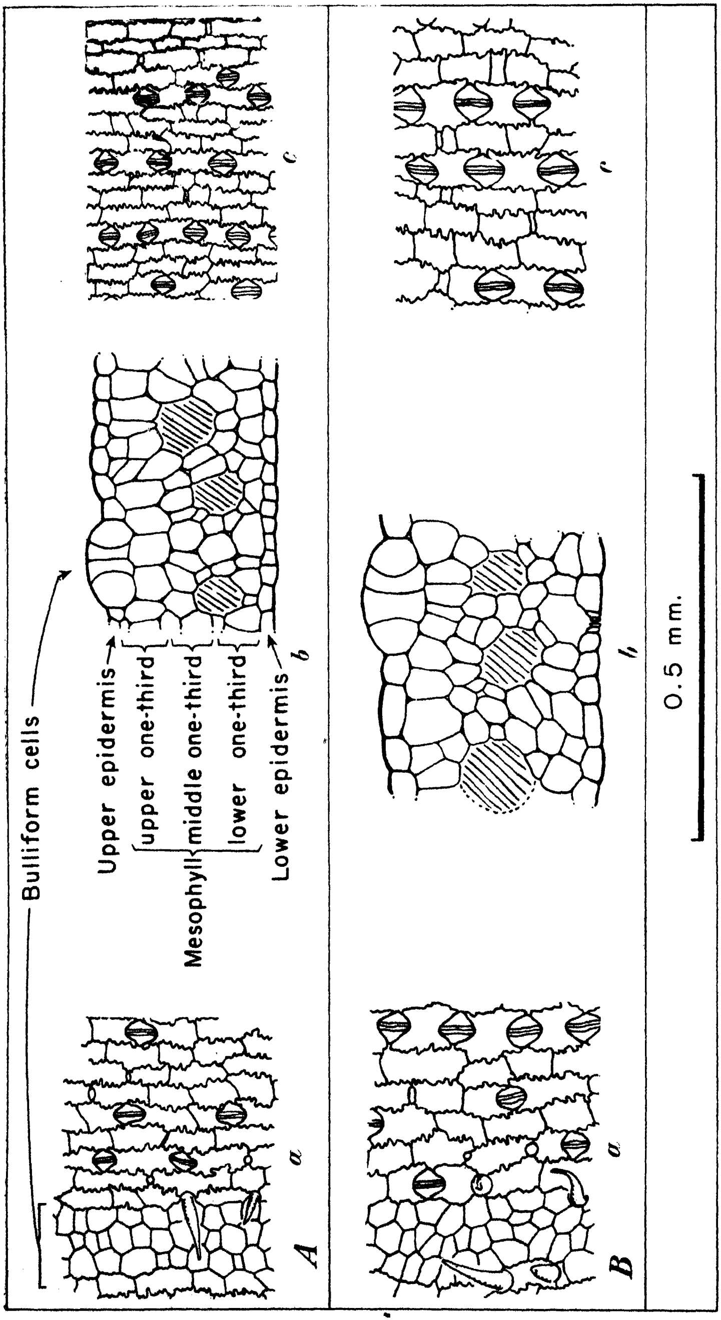


FIGURE 5.—Relative dimensions of cells in the epidermis and mesophyll of diploid (*A*) and tetraploid (*B*) maize. *a*, Upper epidermis, face view; *b*, cross section; *c*, lower epidermis, face view. Camera lucida drawings. Cross hatching indicates vascular bundles.

midrib) than they are thick (in dimension perpendicular to the leaf surface). The upper epidermis is provided also with long rows, several cells in width, of essentially isodiametric and somewhat distended bulliform cells. Along the edges of these areas of bulliform cells are occasional unicellular, conical hairs. Stomata are interspersed in certain rows of the epidermal cells. The lower epidermis resembles the upper except that bulliform cells are absent, the stomata are more abundant, and the cells tend to be smaller than in the upper epidermis. The mesophyll is composed of relatively unspecialized parenchyma cells, which differ markedly in size and have only occasional, very small intercellular spaces. Vascular bundles of various sizes are located within the mesophyll.

For the determinations of cell number and cell size the mesophyll was subdivided for convenience into the upper, middle, and lower thirds. The cells of the lower and middle thirds were of about the same size, but the presence of numerous vascular bundles tended to modify the form of the cells in the middle third. Those of the lower third were more nearly symmetrical. The cells of the upper third of the mesophyll were larger than those of the middle and lower third. Strain D was used for the most complete series of measurements of the cells of the leaf, since the relative length, width, and thickness of the diploid and tetraploid leaves of this strain most nearly approximated the averages for all of the strains that were studied (tables 3 and 4). Strain E was used in a few cases for an additional comparison. The cell measurements were made so as to avoid vascular bundles and bulliform cells. In measuring the length and width of the epidermal cells, groups of 8 to 12 cells from cell rows containing no stomata were measured as a unit to reduce experimental error. Elsewhere, individual cells were measured. The epidermal cells were measured with an ocular micrometer from hand sections. The mesophyll cells were measured from projected drawings of frozen microtome sections.

The mesophyll averaged 5.4 cells in thickness in the diploid and 6.2 cells in the tetraploid, the determinations being based on counts from 45 sections taken in the region of the leaf 15 cm. from the base and midway between the midrib and the margin. It is probable that this difference in cell number was not significant. The mesophyll in the third leaf of the sectorial chimera previously mentioned averaged 3.8 cells in thickness for the tetraploid sector and 3.4 for the diploid sector, indicating that there was no significant difference between the diploid and the tetraploid with respect to the number of cells in the cross-section area of the mesophyll tissue. These data from the chimeral leaf also suggest that the earlier formed leaves of the maize plant have fewer mesophyll cells in cross section than do the later leaves. This fact emphasizes the importance of comparing morphologically homologous organs in quantitative analyses of form differences.

The length and width of the cells of the lower epidermis were measured at distances of 15 and 30 cm. from the base of the leaf blade in strains D and E (table 5) to determine the relation of location along the longitudinal axis of the leaf to cell size. A series of measurements were made also at 50 cm. from the base in strain D to check further on the position effect (table 5). It was immediately evident that the length and width of the lower epidermal cells fluctuated markedly with their position in the leaf. Thus, in strain D the absolute length of the cell, in both the diploid and the tetraploid, increased with the distance between the cell and the base of the leaf. This was true also in the diploid leaf of strain E, but the reverse was the case in the tetraploid leaf of strain E. The width of the lower epidermal cells did not change appreciably with position in strain E, but it did vary somewhat in strain D, although not in any very significant manner. The length of the epidermal cells appears to change with position in the leaf to a greater absolute extent than does their width. Chromosomal constitution likewise has a major effect on the dimensions of the epidermal cells.

TABLE 5.—*Dimensions of cells in epidermis and mesophyll of the tenth leaf in diploid and tetraploid maize*

Tissue, cell dimension, strain, and distance above ligule (cm.)	Diploid (2*n*)			Tetraploid (4*n*)			Ratio of cell dimension (4*n*/2*n*)
	Average cell dimension and standard error	Cells measured	Leaves sampled	Average cell dimension and standard error	Cells measured	Leaves sampled	
Lower epidermis:							
Length [1]—	μ	*Number*	*Number*	μ	*Number*	*Number*	
D (15)	73±1	2,108	10	82±3	1,837	10	1.12
E (15)	83±3	873	5	101±5	730	5	1.22
D (30)	87±4	556	2	98±8	506	2	1.13
E (30)	98±2	492	2	88±3	553	2	.90
D (50)	104±4	154	1	95±3	168	1	.91
Width [2]—							
D (15)	44±2	1,900	10	44±2	1,900	2	1.00
E (15)	34±1	900	5	47±1	900	5	1.38
D (30)	49±1	600	2	46±1	600	2	.94
E (30)	36±1	600	2	47±1	600	2	1.31
D (50)	30±1	200	1	42±1	200	1	1.40
Thickness [3]—D (15)	31±1	250	5	37±1	250	5	1.19
Upper epidermis:							
Length [1]—							
D (15)	75±2	1,073	5	73±3	1,049	5	.97
D (50)	90±3	178	1	117±3	137	1	1.30
Width [2]—							
D (15)	36±2	1,000	5	49±2	1,000	5	1.36
D (50)	32±1	200	1	42±1	200	1	1.31
Thickness [3]—D (15)	39±2	50	5	48±2	50	5	1.23
Mesophyll:							
Upper third:							
D (15)—							
Width [2]	48±5	100	5	59±5	100	5	1.23
Thickness [3]	51±3	100	5	69±2	100	5	1.35
Middle third:							
D (15)—							
Width [2]	41±3	100	5	43±2	100	5	1.05
Thickness [3]	44±2	100	5	47±1	100	5	1.07
Lower third:							
D (15)—							
Width [2]	43±3	100	5	41±2	100	5	.95
Thickness [3]	45±3	100	5	48±3	100	5	1.07

[1] Diameter parallel to surface and midrib of leaf.
[2] Diameter parallel to surface and perpendicular to midrib of leaf.
[3] Diameter perpendicular to surface of leaf.

In strain D there was a definite tendency for the length of the cells in the upper epidermis to increase and for their width to decrease with their distance from the ligule, or leaf base (table 5).

The relative cell dimensions (4*n*/2*n*) varied with position in strains D and E. With respect to the length of lower epidermal cells there was a tendency for the cells of the tetraploid to be relatively longer than those of the diploid near the base of the leaf in both D and E, although the ratios were by no means the same in the two strains. However, with respect to cell width there was not even this much agreement between strains D and E. Both chromosomal constitution and position in the leaf had a varying relation to the ratio between cell length and cell width in the lower epidermis of the diploid and tetraploid leaves. Cell thickness in the lower epidermis at a distance of 15 cm. from the ligule was somewhat less than cell width in the tetraploid leaves and 1.19 times cell width in the diploid leaves.

The relative dimensions of the cells in the upper epidermis also varied, depending on position in the leaf, the length of the cells being nearly the same in the diploid and tetraploid leaves at 15 cm. from the base of the leaf but 1.3 times as long in the tetraploid as in the diploid at a distance of 50 cm. from the base. The width of the cells in the tetraploid was somewhat more than 1.3 times that in the diploid, both at 15 cm. and 50 cm. from the base. The thickness of the upper epidermal cells at 15 cm. from the base was 39μ in the diploid and 48μ in the tetraploid, the $4n/2n$ ratio being 1.21.

In the mesophyll the cells of the lower and middle thirds of the leaf were very nearly isodiametric and tended to be the same size or but slightly larger (0.95 to 1.07 times) in the tetraploid than in the diploid. The cells of the upper third of the mesophyll were, however, not only markedly larger than the rest of the cells of the mesophyll in the same leaf but also relatively much larger in the tetraploid leaves than in the diploid leaves, the ratio being 1.35.

The survey of cell size in the various parts of the diploid and tetraploid leaves of strains D and E showed a marked tendency toward independent dimensional behavior of each of the parenchymatous constituents of the leaf, and there was a pronounced variation in cell dimensions both in comparable regions of different strains and in different regions of the same strain. Although there was a tendency for the cells of a given tissue in the tetraploid leaf to be larger than the comparable cells in the diploid leaf, in another strain or in another location in the leaves of the same strain the cells of the tetraploid leaf in some cases were of the same size or even slightly smaller than the comparable cells of the diploid leaf. Furthermore, the relative size of all the cells contributing to a given dimension of a leaf was not changed in the tetraploid to precisely the same degree as the gross dimensions of the leaf were changed with respect to the comparable dimensions of the diploid leaf. Thus the length of the leaf, although it remained essentially the same in the tetraploid and diploid leaves of strain D, nevertheless represented the summation of fewer cells near the base and middle of the lower epidermis but smaller and more cells toward the outer region of the leaf of the tetraploid as compared with the diploid. Width of leaf in the tetraploid plants of strain D was 1.21 times that of diploid plants in the same strain (table 3), yet the lower epidermis had cells of the same width in the tetraploid as in the diploid at distances of 15 cm. and 30 cm. from the base of the leaf (table 5), so there must be more cells in that region of the tetraploid; whereas at 50 cm. from the base, the lower epidermis of the tetraploid leaf had fewer cells in width than did the diploid. The upper epidermis, on the other hand, had larger but fewer cells in width than would be expected from the external measurements of the leaf. The thickness of the leaf in tetraploid plants of strain D was 1.33 times that of the diploid, yet the lower epidermal cells were only 1.19 times as thick, the lower and middle mesophyll cells only 1.07 times as thick, the upper third of mesophyll cells 1.35 times as thick, and the upper epidermal cells 1.23 times as thick. In spite of the independence of the various cell layers, insofar as relative dimensions and rates of division were concerned, there was no visible evidence of tissue tension. The changed pattern of the leaf in the tetraploid was apparently as well integrated during development and at maturity as in the original diploid form, which suggests that the organ as a whole in some obscure manner exerts a regulatory influence over its constituent parts during ontogeny.

ESTIMATES OF TOTAL NUMBER OF CELLS IN THE MATURE LEAF BLADE

Having determined the over-all dimensions of the mature tenth leaf blade and the dimensions of individual cells in representative regions of the leaf, exclusive of the vascular tissue (table 5), in various diploid and tetraploid stocks of maize (tables 3 and 4), it was possible to estimate with a fair degree of accuracy the total number of cells throughout the various tissues of the leaf. The summation of these values would then yield an estimate of the total number of cells, exclusive of the vascular tissue, in the entire diploid and tetraploid leaf blades. So far as the writers are aware, suitable data have not previously been available from which reliable estimates of the total number of cells in the leaves of the maize plant could be made. The diploid and tetraploid stocks of strain D were utilized in making these computations, as the cell and organ dimensions in this strain closely approximated the mean for all of the strains studied. Cell dimensions at 15 cm. from the base of the leaf blade were utilized in making the computations of cell number, as a complete set of values for other regions of the leaf was not available.

Table 6 records the number of cells in the various tissues throughout the length of the diploid and tetraploid leaf blades, calculated from the dimensions obtained for the leaf blade and its constituent cells.

In making these computations it was necessary to assume that the mesophyll cells had the same length and width and that each of the three regions of the mesophyll was two cell layers thick. The cytological observations of the mesophyll tissue indicated that these were reasonable assumptions.

Table 6 also lists the number of cells in the maximum width of the various tissues of the mature leaf, calculated from the dimensions obtained for the leaf blade and its constituent cells.

The relative values for the different tissues of the diploid and tetraploid leaves were not the same. For example, there were fewer cells in the lateral extent of the upper epidermis of the tetraploid leaf than there were in the upper epidermis of the diploid leaf; but the reverse was true of the lower epidermis. This was due to the fact, previously emphasized, that different tissues often respond differently with respect to the changes in cell dimensions that result from chromosome doubling.

Leaf thickness, which was consistently 1.35 times as great in the tetraploid as in the diploid (table 4), is a dimension in which, by direct count, there was essentially the same number of cells in both stocks (7.4 ± 0.3 in the $2n$ and 8.2 ± 0.3 in the $4n$, based on counts of 45 different diameters in each).

TABLE 6.—*Number of cells in mature tenth leaf blade of diploid and tetraploid maize*

CELLS IN LENGTH OF LEAF

Leaf tissue	Cell rows	Cells in—	
		Diploid	Tetraploid
	Number	*Number*	*Number*
Upper epidermis	1	11,000	11,300
Mesophyll:			
Upper third	2	34,400	27,900
Middle third	2	40,300	38,200
Lower third	2	38,400	40,100
Lower epidermis	1	11,300	10,000
Total	8	135,400	127,500
CELLS IN WIDTH OF LEAF			
Upper epidermis	1	2,500	2,200
Mesophyll:			
Upper third	2	3,800	3,700
Middle third	2	4,400	5,100
Lower third	2	4,200	5,400
Lower epidermis	1	2,100	2,500
Total	8	17,000	18,900
TOTAL CELLS IN LEAF TISSUES			
Upper epidermis	1	14,000,000	12,000,000
Mesophyll:			
Upper third	2	33,000,000	26,000,000
Middle third	2	44,000,000	49,000,000
Lower third	2	40,000,000	54,000,000
Lower epidermis	1	12,000,000	13,000,000
Total	8	143,000,000	154,000,000

The entire number of cells in the mature leaf blade may be computed from the foregoing data by assuming that the leaf blade is a wedge-shaped solid. The total number of cells in any tissue would then be half of the number in a rectangular solid with a corresponding number of cells in each linear dimension. On this basis, cell number in each of the tissues and the total in the whole leaf may be determined. The number of cells (to the nearest million), computed in this fashion, is shown in table 6.

The above results may be checked by taking half the product of the average number of cells in length, width, and thickness. This gives (to the nearest million) 144 million cells for the diploid and 150 million for the tetraploid, the difference between these results and those reached by the summation method being due to the way the figures have been rounded off in the process of calculation.

It is apparent from these figures that the diploid and tetraploid leaves contained approximately the same number of cells, the total number of cells in the diploid being 143 million and in the tetraploid 154 million. Considering the methods used in arriving at these estimates, the slightly larger number of cells in the tetraploid is probably not significant. These data on cell number indicate that there was a marked tendency for the diploid and tetraploid leaf blades to undergo approximately the same number of cell divisions during ontogeny, but probably at a slower rate in the tetraploid.

As the number of cells in the diploid and tetraploid leaves is essentially the same, the average volumes of the cells would have the same relationship as would the volumes of the respective mature leaves. Applying the fundamental formula used above, namely, $\frac{\text{length} \times \text{width} \times \text{thickness}}{2}$, the mean volume of the diploid leaf blade was found to be 9,800 cubic millimeters and that of the tetraploid 15,800 cubic millimeters. Thus the volume of the tetraploid leaf blade was 1.6 times that of the diploid and therefore cell volume in the tetraploid blade averaged 1.6 times that of the diploid. In this connection it should be recalled that there was a considerable diversity of cell-size relationships in the diploid and tetraploid leaf tissues in various regions of the blade. However, the pattern of the tetraploid leaf was relatively constant and differed significantly from that of the diploid in that it was consistently wider and thicker but of the same length. Apparently, cell-number and cell-size relations were adjusted somewhat differently in different regions of the leaf to conform to the modified leaf pattern of the tetraploid plant. These mature-leaf comparisons also indicate that the cells of the mature tetraploid leaf were relatively not as much larger than those of the diploid as they were at the time of initiation of the leaf in the shoot apex, although absolute size in each increased greatly during ontogeny.

COMPARATIVE RELATIONS OF DIPLOID AND TETRAPLOID SHOOT APICES AND LEAVES

Various relations between the diploid and tetraploid shoot apices and leaf blades are summarized in table 7. In these comparisons the diploid was taken as the standard of comparison (i. e., $2n$=unity), and the extent to which the tetraploid differed from it ($4n/2n$) was indicated for a number of different characteristics. The ratios, presented along with the raw data in preceding tables, are here translated into more general terms. These ratios were computed from the mean values given in the tables. Since these mean values have a range of variation, as indicated in their standard errors, the ratios derived from them are to be interpreted as arbitrary expressions of a similar range of variation. In preparing table 7, ratios falling near 1.26 were grouped together in one column (column 4, "$4n$ markedly greater than $2n$"); ratios falling near 1.00 were similarly grouped in another column (column 2); intermediates in still another column (column 3); and those much greater than $2n$ were grouped in the last column. (See footnotes 2, 3, 4, and 5, table 7.)

The group of ratios centering about the linear value 1.26 (column 4, table 7) is especially noteworthy, since this value represents the expected deviation of the tetraploid from the diploid when the only modification of the tetraploid is a doubling of volume, with the result that linear dimensions are increased by $\sqrt[3]{2}$, or 1.26. This condition may be referred to as the "ideal gigas" state, since it is essentially the state that is commonly attributed to gigas plants.

The concept of an "ideal gigas state" is very useful and may well be defined in more detail at this point. The ideal gigas state is one in which (1) the tetraploid organ or other structural unit has the same number of cells and cells of the same shape as the diploid; but (2) the volume of the constituent cells is doubled in the tetraploid in

TABLE 7.—*Linear relation (or equivalent* [1]*) between tetraploid and diploid ($4n/2n$) shoot apices and leaves*

Item (1)	$4n$ approximately equal [2] to $2n$ (2)	$4n$ slightly greater [3] than $2n$ (3)	$4n$ markedly greater [4] than $2n$ (4)	$4n$ much greater [5] than $2n$ (5)
Origin of leaf blade				
Shoot apex				
Gross dimensions (length, width, area, volume)			×	
Cell dimensions (width, area, volume)			×	
Nuclei dimensions (width, volume)			×	
Leaf initial, lateral extent			×	
Mature leaf blade				
Gross dimensions (mean for all 5 stocks)				
Length	×			
Width		×		
Thickness			×	
Volume		×		
Cell dimensions [6]				
Length				
Upper epidermis		**15D**		**50D**
Lower epidermis		50D; **30E**	**15D**; 30D; 15E	
Width				
Upper epidermis			50D	**15D**
Mesophyll				
Upper third			**15D**	
Middle third		**15D**		
Lower third	**15D**			
Lower epidermis	**15D**; 30D	15E	30E	50D
Thickness				
Upper epidermis			**15D**	
Mesophyll				
Upper third				**15D**
Middle third		**15D**		
Lower third		**15D**		
Lower epidermis			**15D**	

[1] Ratios between areas are represented by their square roots; between volumes by their cube roots.

[2] 0.89 through 1.03, mean 0.96.

[3] 1.04 through 1.18, mean 1.11.

[4] 1.19 through 1.33, mean 1.26.

[5] Over 1.33.

[6] The number indicates the distance in millimeters from the base (ligule) of the leaf blade; the letter designates the strain.

proportion to the doubling of the volume of the nucleus, due in turn to the doubling of the number of chromosomes. The result of (1) and (2) is that (3) the external form of the tetraploid structural unit is the same as that of the diploid, but there is an increase in its size such that the total volume of the tetraploid is doubled, its surface area is greater by $\sqrt{2}$, or 1.41 times, and its linear dimensions are greater by $\sqrt[3]{2}$, or 1.26 times. This establishes a convenient, although admittedly arbitrary, definition of the morphological nature of the ideal gigas structure. The definition is based on the assumption that differences in form between tetraploids and diploids may be traced very simply to a mechanical doubling of nuclear volume and that developmental relations are not otherwise affected by doubling the number of chromosomes and genes. This definition ignores the possibility that the fundamental size relation between cell and nucleus may be the relation of volume to area. Also ignored are (1) the possible influence of absolute cell size on the realization of genically controlled form and (2) the altered functional activity of the genes when present in the doubled number.

The state in which the tetraploid closely approximates the diploid (column 2, table 7) represents a maintenance in the tetraploid of the condition typical of the diploid and is the least likely expectation when dimensions are being compared, if mechanical increase in cell size is the only biologically significant difference between the tetraploid and diploid plants.

It is apparent from table 7 that the ideal gigas state is closely approached in the shoot apex; but in the mature leaf, departure from the ideal gigas state is characteristic of the various tissues of the leaf blade and the relation between the diploid and tetraploid cell dimensions is extremely variable. For example, in strain D, at 15 cm. from the ligule (see values indicated in boldface in table 7), cell width in the tetraploid leaf blade was approximately equal to that of the diploid in the lower epidermis and in the lower third of the mesophyll; in the middle third of the mesophyll cell width was slightly greater in the tetraploid than in the diploid; in the upper third it was markedly greater in the tetraploid; and in the upper epidermis it was much greater in the tetraploid than in the diploid. Similarly for other cell dimensions the tissues in the same and different regions of the diploid and tetraploid leaves varied widely and in a wholly unpredictable manner. In the fully differentiated tissues of the mature leaf there was no consistent relation between the dimensions of the organ and its constituent cells such as existed in the undifferentiated tissues of the shoot apex. These observations suggest that the cell exerts a more potent influence in determining organ pattern in the embryonic shoot apex than it does in the more highly specialized leaf blade.

DISCUSSION

The fact that the mature tetraploid organ seldom has the perfect gigas form as here defined has already been emphasized by several investigators. Von Wettstein (*13*) described in detail the variability in morphological constitution, not only from "Sippe" to "Sippe" on a genetic basis but also from "Rasse" to "Rasse" on a valency basis, within each of a number of moss species. He stated that the quantitative changes in chromosome mass of the original cells result in the most diverse morphological changes, often out of proportion, which can only be demonstrated on an experimental basis to be the result of increase in chromatin mass. In a study of members of the polyploid series in *Datura stramonium*, which was limited chiefly to a consideration of mature organs, Sinnott, Houghtaling, and Blakeslee (*12*) concluded that the primary differences between the members of the series were due to an increase in cell size associated with an increase in chromosome number, but they found many deviations from the perfect gigas state in specific tissues. Müntzing (*6*), in reviewing and correlating the large number of isolated cases described in the literature on polyploidy up to 1936, pointed out that autotetraploids are in general larger and have larger cells but that the differences are not quantitative, that is, the mature organs are not typically gigas in form. Polyploidy has not been studied extensively in animals, but an extremely interesting series of polyploid larvae in the newt *Triturus* was described by Fankhauser (*5*). The larvae of autotriploid newts had the same gross dimensions as the diploids, but they had larger and, except in some tissues, fewer cells. This tendency of the triploid to maintain the proportions of the diploid was attributed by Fankhauser to some phenomenon of regulation whose mechanism is at present unknown. None of these workers made ontogenetic comparisons of the tetraploid and diploid or studied the relation of the embryonic tissues to the ultimate form of the organs derived from them.

The development of the leaf in diploid and tetraploid maize was investigated by Abbe, Randolph, and Einset (*1*), who showed that for any given length the tetraploid leaf was broader than the diploid. They showed also that this condition was related to the greater size of the tetraploid shoot apex, this greater size in turn being due to larger cells rather than to more cells. Only the tenth leaf blade was studied in relation to the shoot apex while the latter was in the tenth plastochron, and it was considered very important that the comparison be restricted to identical plastochrons because of changes in absolute and relative dimensions through successive plastochrons. This viewpoint has recently been elaborated more fully by these writers (*2*). Subsequently, Sinnott, Blakeslee, and Franklin (*11*) studied the influence of tetraploidy on form in the ontogeny of the fruit of four strains of cucurbits in which genetically comparable tetraploid and diploid races were available. They found that the tetraploid in two of the four strains had larger fruit primordia, and in all four strains the volume of the cells in the primordia was doubled, although at maturity the size relations of the tissues and fruits varied from line to line.

In a comparison of tetraploid and diploid shoot apices of *Vinca rosea*, Cross and Johnson (*3*) reported recently that the increased size of the tetraploid apex was due to an increase in cell width unaccompanied by any significant increase in cell thickness. Dr. Cross[3] very kindly provided the additional and pertinent information that the shoot apices listed by Cross and Johnson (*3*, *table 1*) were all in the fourth plastochron, except diploid apex "4," which was in the fifth plastochron, and that the original diploid strain had been inbred for 14 years. The quantitative data included (*3*, *table 1*) should therefore be recognized as representing thoroughly comparable material, both morphologically and genetically.

It is apparent from these developmental studies of the effects of polyploidy on the form and cellular organization of mature organs and the primordia from which they originate that the morphological consequences of chromosome doubling are variable and presumably depend upon the genotype involved in particular strains or species. In this recently developed field of morphogenetics it seems unwise to attempt generalizations until additional observations are available, but in quantitative studies of this sort one cannot overemphasize the importance of obtaining adequate data from strictly comparable diploid and tetraploid material that is genetically homogeneous and morphologically homologous.

The various degrees of departure from the ideal gigas state in the mature leaf blade of the writers' material raise a number of considerations: (1) The relation of cell number to organ size; (2) the relation between growth rate and the rates of increase in cell number and cell size; (3) the possible influence of absolute cell size on the rate of cell division; (4) the development of functional specialization of the maturing tissues of the leaf; and (5) evidence of genetic modification of any tendency to maintain the ideal gigas state throughout ontogeny.

---

[3] Correspondence, January 7, 1942.

Cell number and cell size as compared with organ size are of significance, since the interrelationship of these factors is a means of defining cell pattern as it contributes to organ form. Thus, in the shoot apex of the tetraploid as compared with that of the diploid, during the tenth plastochron, there is the same number of cells in median section and in basal diameter. Furthermore, cell volume is practically doubled in the tetraploid shoot apex. Therefore, the pattern of cell arrangement and the proportions of the cells characteristic of the diploid have been retained in the tetraploid. The tetraploid also retains the same general external form of the shoot apex as the diploid. The shoot apex of the tetraploid thus serves as an excellent and diagrammatic example of a perfect gigas organ. Because of its intimate relationship with the shoot apex, the leaf primordium may be assumed to share with the shoot apex this perfect gigas character. However, when the mature leaf blade is analyzed in a similar manner, it is apparent that the tetraploid is very different from the diploid with respect to both external form and the dimensions of the cells comprising the various tissues of the leaf.

There is no evidence that the tetraploid leaf in itself is abnormal structurally or functionally, since in relation to the diploid leaf it displays no consistent change in its cellular constitution or general form. Starting with the length of the leaf, one finds that the mature tetraploid and diploid leaves tend to be of essentially the same length in most of the stocks studied, but the tetraploid leaf is consistently thicker and is usually broader than the diploid leaf. Furthermore, the cell pattern of the diploid leaf is not consistently retained in the tetraploid for any of the regions of either the upper or lower epidermis, nor is there a general agreement between different stocks in the manner in which the diploid pattern fails to be maintained in the tetraploid. Although the diploid and tetraploid leaf blades differed significantly in external form with respect to the total number of cells, the mature tetraploid leaf blade displayed perfect gigas characteristics, in that it had the same number of cells as the diploid; but in other respects the requirements of the perfect gigas state were not met. As is evident from table 7, the constituent cells of the tetraploid leaf blade did not have the same proportions as the corresponding cells of the diploid. Furthermore, the total volume of the tetraploid mature leaf blade was not double that of the diploid but was only 1.6 times that of the diploid. Since the volume of the tetraploid mature leaf blade was 1.6 times that of the diploid and the number of cells was essentially the same in both, it is evident that the volume of the hypothetical "average" cell in the tetraploid was 1.6 times that of the diploid. Thus the second requirement of the perfect gigas state, namely, that the volume of the cells be doubled, also failed to be realized in the mature tetraploid leaf blade. The interaction of these factors is such that the external form of the tetraploid also fails to meet the requirements of the ideal gigas state, in that the individual linear dimensions of the tetraploid were not 1.26 ($\sqrt[3]{2}$) times those of the diploid leaf blade.

It is evident that, except for the number of cell divisions which contributed to leaf thickness, the cell-division activity from plane to plane and from tissue to tissue in the tetraploid differed from that of the diploid. This difference existed also in the degree of cell enlargement from diameter to diameter and from tissue to tissue, since in these characteristics there was no evident simple relationship between tetraploid and diploid. Obviously, the relation of the growth rates of the respective leaf blades to the rates of increase in the number and size of their constituent cells was extremely variable in the different parts of the leaf blade.

Since the growth rate of the tetraploid leaf blade was slower and the initiation of maturation later, it is apparent that, with the same number of cells in the mature tetraploid and diploid leaves, the rate of cell division must have been correspondingly slower in the tetraploid.

The problem of the rate of increase in average cell size cannot be dealt with in this simple manner. It is evident, however, that the tetraploid cells reach a greater size before dividing than do the diploid cells; and since the growth rate of the tetraploid leaf blade as a whole was slower than that of the diploid, it is logical to assume that the rate of increase in the volume of the individual tetraploid cells is slower than that in the diploid cells.

If one accepts as valid the well-known concept that an optimum surface-volume relation is essential to normal growth, it may be inferred that the greater absolute size of the cell and nucleus in the tetraploid has had a major influence in reducing the rate of growth and the ultimate size of the organs attained by the tetraploid plant. Since the volume of the tetraploid cell increased as the cube of the linear dimensions of the diploid, and the area of its limiting membrane increased merely as the square of these same dimensions, as a result of chromosome doubling, some degree of metabolic unbalance might be expected during the development and differentiation of the tetraploid, especially in the more highly differentiated organs, such as the leaf blade. The concept is a familiar one and needs no further elaboration in the absence of a specific and detailed factual basis in our material; yet it cannot be ignored as an important physiological factor which may be of potential major significance in the economy of the polyploid plant and which may be held indirectly responsible, at least in part, for the more sluggish growth rate of the tetraploid leaf blade.

With the increasing functional specialization of the maturing tissues of the leaf blade, there is ample opportunity for many diverse factors to interact in a manner to account for the notable lack of uniformity between the quantitative behavior of the individual cells and of the leaf as a whole. This lack of uniformity in the form and cell relations of the mature leaf is in such striking contrast to the uniformity throughout the unspecialized shoot apex that the coincidence between functional specialization and lack of uniformity seems to assume a probable cause-effect relationship.

Genetic constitution may also play a major role in modifying the quantitative response to the mechanical increase in nuclear size, not only, as just suggested, within the individual genotype as ontogeny proceeds, but also from genotype to genotype. Certainly there is variation of a significant nature between cell size and organ dimensions from genetic stock to genetic stock, as indicated in tables 3, 4, and 5. It is clearly apparent that the cells of the same tissue or of the same leaf dimension in the tetraploid differ in the degree of their departure from the diploid condition, depending on the genotype in which doubling has occurred. This fact may be interpreted as evidence that the ultimate effect of doubling the number of genes through doubling the number of chromosomes is not always arithmetic in terms of cell or organ volume at maturity. Yet the shoot apex, both in cell and organ size, has responded to tetraploidy almost exclusively by the doubling of volumes. The genic complement, or genome, of the cells is the same in the shoot apex as in the mature leaf, yet these two organs respond differently to chromosome doubling.

A relatively simple explanation of this situation would be to assume that different genes belonging to the same genome, operating at different stages in the life cycle and responding differently to a reduplication of their number, are responsible for the observed differences in the effect of chromosome doubling on the shoot apex and on the mature leaf blade. The genes controlling the size, shape, and cell-pattern relations of the shoot apex in maize responded in a similar manner to chromosome doubling, with the result that the tetraploid shoot apex was enlarged symmetrically and attained the ideal gigas state. But this was not true in the tetraploid shoot apices of *Vinca* studied by Cross and Johnson (*3*), in which chromosome doubling produced an increase in cell width but had no effect on other dimensions of the cells. However, the doubling of the genes concerned with the development of the pattern of the mature maize leaf blade resulted in responses that produced alterations in the symmetry of the pattern as it existed in the diploid plants, and the end result of these alterations was a tetraploid leaf pattern that had no simple arithmetical relation to the original diploid pattern.

With respect to the shoot apex in the genetic stocks that were studied in this investigation, the doubling of cell size and organ size in the tetraploid appeared to be correlated directly with the doubling of the number of chromosomes, and there were no departures from this condition that might be attributed to the nonuniform action of specific genes. However, in the mature leaf there was a conspicuous lack of uniformity in the response of the organ as a whole and of its constituent cells and tissues to chromosome doubling, both in different regions of the same leaf and in the leaves of different stocks. This suggests that certain genes belonging to particular genomes responded differently to chromosome doubling. The cumulative or quantitative action of certain genes, and its absence in others, would account for the observed differences between the various diploid and tetraploid maize stocks with respect to leaf morphology.

Similar differences in the response of certain genes to chromosome doubling presumably resulted in percentage increases in the carotenoid content of certain strains of tetraploid maize and percentage decreases in other strains analyzed by Randolph and Hand (*10*). Likewise, the decrease in stature and fertility that accompanies chromosome doubling in inbred strains of maize and the absence of these deleterious effects when the chromosomes of hybrid stocks are doubled, which was interpreted by Randolph (*8*) as an indication that heterozygosity per se may be an important cause of hybrid vigor, are explainable on a genic basis in terms of similar disharmonious types of quantitative gene action. Unfortunately, the genic analysis required to validate these suggested interpretations is not easy to obtain. The inheritance of such characters is presumably quantitative, and tetraploidy imposes further complications.

An interpretation of the morphological significance of these observations involves not so much a comparison of individual dimensions as the relation between dimensions, this being the basis of form. As already stated, the form of the tetraploid and diploid shoot apices was essentially the same during the period of initiation of the tenth leaf primordium (i. e., during plastochron 10), but the tetraploid was twice the volume of the diploid. The basal circumference of the tetraploid approximated the expected value of $\sqrt[3]{2}$ times that of the diploid. Throughout the development of the tenth leaf blade, its width for any given length was practically as much greater in the tetraploid than in the diploid as the tetraploid shoot apex was greater in basal circumference, and therefore as the tetraploid leaf primordium was greater in width. The original advantage in width given to the tetraploid leaf blade by its origin from a larger shoot apex was maintained throughout ontogeny.

An analogous situation was described by the writers (*1*) in the ontogeny of successive leaves of the diploid plant. The shoot apex in the ontogeny of the diploid became increasingly larger in each successive plastochron, and the leaf blade originating in each plastochron was as much wider at any given length than the preceding one as the shoot apex was greater in basal circumference than the preceding one; that is, in the ontogeny of the diploid there is an increase in size of leaf initial and this advantage is maintained throughout ontogeny. In the case of the ontogeny of the diploid, the increasing size of the shoot apex was due primarily to an increase in the number rather than in the size of the constituent cells. The comparison of the tetraploid and diploid shows that the leaf which is wider for any given length originates from the larger shoot apex. But in this case the shoot apex was larger because of larger cells and not because of more cells.

There was a change in the form of successive leaves in the ontogeny of the diploid, and there was a similar difference in the form of the tetraploid and diploid leaves. Both changes in form were correlated with a similar change in form of the shoot apex. But the shoot apex in the ontogeny of the diploid changed in form because of an increase in number of cells, whereas that of the tetraploid changed because of an increase in cell size. In neither can the change of form be attributed to a difference in the quality of the genome. In the ontogeny of the diploid the change is a characteristic expression of the diploid genome through its influence on rate of cell division in the shoot apex, on the one hand, and of rate of initiation of new leaf primordia on the other. Cells multiplied in number in the shoot apex more rapidly than leaf initials were set off in successive plastochrons, so that the shoot apex became increasingly larger and, therefore, the successive leaf initials also became larger. There was no basic difference in the manner in which growth in width was correlated with growth in length in the ontogeny of successive leaves in the diploid, the wider the leaf initial the wider the developing leaf, but this was obviously not due to a change in the genetic constitution of the plant. Similarly, there is no qualitative difference in the genetic constitution of the tetraploid and diploid plants.

The greater size of the tetraploid shoot apex can be traced directly to a mechanical increase in nuclear size due to the presence of twice as many chromosomes as are present in the diploid. This change did not modify the number of cells in the shoot apex during the tenth plastochron. Therefore, the greater width for any given length of the tetraploid leaf blade can be considered a residual effect of changed nuclear size in the shoot apex. Thus we have two mechanisms, nongenetic in nature, that may result in changes in the form of the leaf blade. In each case the changes may be traced directly back to the gross size of the shoot apex. In one case the size of the shoot apex changes because of a change in the number rather than in the size of the constituent cells (ontogeny of $2n$); in the other case, because of a change in the size rather than in the number of cells ($4n$ vs. $2n$).

The changes in the developmental morphology of the leaf blade resulting from chromosome doubling are of such a nature at different periods of ontogeny that there is a basis for distinguishing between the direct effect of increasing nuclear volume on cell size and organ size and the indirect effect of doubling the number of specific genes. Earlier in the development of the tetraploid maize plant the mechanical effect of doubling the number of chromosomes seems the more important, and there is a residual effect of this which may be observed in subsequent development. Increasingly important in later development are the morphological effects evident in the departure of cells and of linear dimensions from the perfect gigas state. This may be attributed, in part at least, to doubling the number of genes. A gene complex that affects a given function (and thus the cell type in which the function is concentrated) may react differently in the doubled state from other gene complexes affecting other functions and, therefore, other tissues. This latter conclusion is based on the varying responses to tetraploidy noted in passing from one region to another in the mature leaf, as well as the varying responses noted in the same leaf region of different stocks.

SUMMARY

A comparative study was made of the shoot apex and tenth leaf in diploid and tetraploid maize, with special reference to the form and structure of the shoot apex during the time of initiation of the tenth leaf primordium (i. e., during the tenth plastochron), the development of the leaf, and the structure of the mature leaf.

The shoot apex of tetraploid plants during plastochron 10 had the same number and arrangement of cells as in the corresponding diploid plants, but the volume of the nuclei and cells was doubled. In consequence of the larger cell size, the tetraploid shoot apex also was correspondingly larger and the tenth leaf initial, which originates as a ridge of tissue that almost completely surrounds the base of the shoot apex, was correspondingly wider than in diploid plants.

The rate of increase in both width and length was slower for the tetraploid than for the diploid leaf blade. Also, growth in length and growth in width were so related that for any given increment in length there was a proportional increment in width; the tetraploid leaf initial was wider at the time of its origin, and this original difference in width for any given length was maintained throughout ontogeny.

At maturity there was a marked tendency in different strains for the tetraploid leaf to be of approximately the same length as the diploid, about 1.16 times as wide, and 1.35 times as thick. The volume of the tetraploid leaf at maturity was thus about 1.6 times

the volume of the mature diploid leaf. The total number of cells in the mature leaf blade was estimated to be about 143×10^6 in the tetraploid and about 154×10^6 in the diploid plants, the difference being of questionable significance. Average cell volume in the tetraploid was about 1.6 times that of the diploid, but detailed analysis of cell size from one tissue region to another indicated failure to maintain this relationship from region to region. The relationship between cell dimensions in the diploid and tetraploid leaf blade was extremely variable in the various tissues in the same region and in different regions of the leaf.

In the fully differentiated tissues of the mature leaf there was no consistent relation between the dimensions of the organ and its constituent cells, such as existed in the undifferentiated tissues of the shoot apex. This suggests that the cell may exert a more potent influence on organ pattern in relatively simple embryonic structures than it does in more highly specialized organs of the mature plant.

The departure of the mature leaf, with its fully differentiated cells and tissues and its marked segregation of functions, from the simple cell-organ relationship characterizing the relatively undifferentiated shoot apex was examined in relation to a number of different factors. Among these were included the rate of increase in cell number and size as related to growth rate, the possible influence of absolute cell size on rate of cell division, surface-volume relations of the cells, the development of functional specialization of the maturing tissues of the leaf, and the evidence of genetic modification of any tendency to maintain the initial effects of chromosome doubling throughout ontogeny.

The concept of an "ideal gigas state" is developed as an aid in the interpretation of the diverse effects of chromosome doubling. This state is attained when the volume of the various structures in the tetraploid, including cells, tissues, and organs, is double that of the diploid, but the relative dimensions of these same structures remain the same; that is, when the external form of the tetraploid structural unit is the same as that of the diploid but its volume is doubled and, as a consequence, the ratio between the surface area of the tetraploid and that of the diploid equals $\sqrt{2}$, or 1.41, and the ratio between the linear dimensions of the tetraploid and those of the diploid equals $\sqrt[3]{2}$, or 1.26. The ideal gigas state was attained in the shoot apex of tetraploid maize, but not in the gross structure and cellular components of the various tissues of the mature leaf.

The significance of doubling the number of chromosomes vs. doubling the number of specific genes in relation to their possible influence on the morphology of tetraploid and diploid maize is discussed. Apparently the relative importance of these two factors differed at different stages in ontogeny. Early in the ontogeny of the shoot apex and leaf primordium, doubling the number of chromosomes resulted in a uniform increase in cell size and organ size with no evidence of differential gene action on individual dimensions. Later in ontogeny, as indicated by the form and structure of the mature leaf, there was abundant evidence of differential gene action. The form of the tetraploid leaf blade was consistently different from that of the comparable diploid in that it was wider and thicker but not longer; and there were also significant form differences between tetraploid stocks of different genotypic constitution and the comparable diploid stocks.

It is suggested that deviations from the ideal gigas state, which were prevalent in the mature leaves and other organs of tetraploid maize, may be due to the cumulative or additive effect of certain genes but not of others, and that the diverse results of chromosome doubling in different stocks of maize, as well as in other organisms, may be due primarily to differences in genotypic constitution.

LITERATURE CITED

(1) ABBE, E. C., RANDOLPH, L. F., and EINSET, J.
1939. DEVELOPMENTAL PATTERN OF THE LEAF BLADE IN 2N AND 4N ZEA MAYS AS RELATED TO THE STRUCTURE OF THE GROWING POINT. (Abstract) Amer. Jour. Bot. 26: 4s.

(2) ——— RANDOLPH, L. F., and EINSET, J.
1941. THE DEVELOPMENTAL RELATIONSHIP BETWEEN SHOOT APEX AND GROWTH PATTERN OF LEAF BLADE IN DIPLOID MAIZE. Amer. Jour. Bot. 28: 778–784, illus.

(3) CROSS, G. L., and JOHNSON, T. J.
1941. STRUCTURAL FEATURES OF THE SHOOT APICES OF DIPLOID AND COLCHICINE-INDUCED, TETRAPLOID STRAINS OF VINCA ROSEA, L. Torrey Bot. Club. Bul. 68: 618–635, illus.

(4) EAST, E. M.
1936. HETEROSIS. Genetics 21: 375–397.

(5) FANKHAUSER, G.
1941. CELL SIZE, ORGAN AND BODY SIZE IN TRIPLOID NEWTS (TRITURUS VIRIDESCENS). Jour. Morph. 68: 161–174, illus.

(6) MÜNTZING, A.
1936. THE EVOLUTIONARY SIGNIFICANCE OF AUTOPOLYPLOIDY. Hereditas 21: 263–378.

(7) RANDOLPH, L. F.
1932. SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE. Natl. Acad. Sci. Proc. 18: 222–229.

(8) ———
1942. THE INFLUENCE OF HETEROZYGOSIS ON FERTILITY AND VIGOR IN AUTOTETRAPLOID MAIZE. (Abstract) Genetics 27: 163.

(9) ——— and FISCHER, H. E.
1939. THE OCCURRENCE OF PARTHENOGENETIC DIPLOIDS IN TETRAPLOID MAIZE. Natl. Acad. Sci. Proc. 25: 161–164.

(10) ——— and HAND, D. B.
1940. RELATION BETWEEN CAROTENOID CONTENT AND NUMBER OF GENES PER CELL IN DIPLOID AND TETRAPLOID CORN. Jour. Agr. Res. 60: 51–64, illus.

(11) SINNOTT, E. W., BLAKESLEE, A. F., and FRANKLIN, A.
1941. A COMPARATIVE STUDY OF FRUIT DEVELOPMENT IN DIPLOID AND TETRAPLOID CUCURBITS. (Abstract) Genetics 26: 168–169.

(12) ——— HOUGHTALING, H., and BLAKESLEE, A. F.
1934. THE COMPARATIVE ANATOMY OF EXTRA-CHROMOSOMAL TYPES IN DATURA STRAMONIUM. Carnegie Inst. Wash. Pub. 451, 50 pp., illus.

(13) WETTSTEIN, F. VON.
1924. MORPHOLOGIE UND PHYSIOLOGIE DES FORMWECHSELS DER MOOSE AUF GENETISCHER GRUNDLAGE. I. Ztschr. f. Induktive Abstam. u. Vererbungslehre 33: 1–236, illus.

DAMPING-OFF IN BROADLEAF NURSERIES OF THE GREAT PLAINS REGION[1]

By ERNEST WRIGHT[2]

Associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

At present there are relatively few pertinent data, in either American or foreign literature, on the etiology of damping-off of broadleaf seedlings, partly because seedling diseases in broadleaf nurseries have been of less importance than those in coniferous nurseries (*1*).[3] However, since the establishment of large Federal nurseries for the production of deciduous stock in 1935, damping-off has assumed considerable importance in the culture of some broadleaf species.

This paper gives the results of some recent field and laboratory studies on the damping-off of broadleaf seedlings in Federal nurseries of the Great Plains region.

LOCATION AND SOIL AND CLIMATIC CONDITIONS OF NURSERIES

To understand fully the damping-off problem involved, it is desirable to consider the location of the nurseries in relation to the soil and climatic conditions of the Great Plains region.

In 1940 there were 17 Federal broadleaf nurseries in the Great Plains, located between latitude 34° and 47° north and longitude 95° and 103° west. Four of the nurseries were maintained by the Soil Conservation Service; the others were operated by the Prairie States Forestry Project. Figure 1 shows the approximate location of these nurseries.

Along the 100th meridian the average annual precipitation in the northern Great Plains is 16–18 inches, whereas in the Panhandle of Texas it is 22–24 inches.[4] Variations in precipitation between northern and southern nurseries were, however, fairly well equalized by higher evaporation losses in the South. During the growing season the average maximum temperature in northern nurseries was 84° F.,

---

[1] Received for publication March 29, 1943. Part of a thesis presented to the faculty of the Graduate College of the University of Nebraska in partial fulfillment of the requirements for the degree of doctor of philosophy, Department of Botany.

[2] This study was initiated under the direction of Dr. George L. Peltier and continued under the supervision of Dr. Robert W. Goss, of the University of Nebraska. The writer gratefully acknowledges this guidance and also expresses his thanks to the following individuals for valuable advice, assistance, and suggestions: Dr. K. F. Baker, Dr. Carl Hartley, Dr. W. J. Himmel, Dr. J. E. Livingston, Dr. K. S. Quisenberry, and Dr. Leva B. Walker. Assistance of the personnel of the Prairie States Forestry Project, Forest Service, and the Soil Conservation Service, of the U. S. Department of Agriculture, is also acknowledged. Greenhouse and laboratory equipment was generously provided by the Department of Plant Pathology of the University of Nebraska.

[3] Italic numbers in parentheses refer to Literature Cited, p. 93.

[4] Data from U. S. Weather Bureau, 40-year period, 1895–1934.

Journal of Agricultural Research, Washington, D. C.

Vol. 69, No. 2
July 15, 1944
Key No. G-1313

whereas in the South it rose to 94°.[5] Wide temperature fluctuations are characteristic of both the southern and northern nurseries during the spring (April to June), when damping-off is most prevalent.

Most of the Federal nurseries in this region were located on the zonal soil group known as the Chernozem, on which the native vegeta-

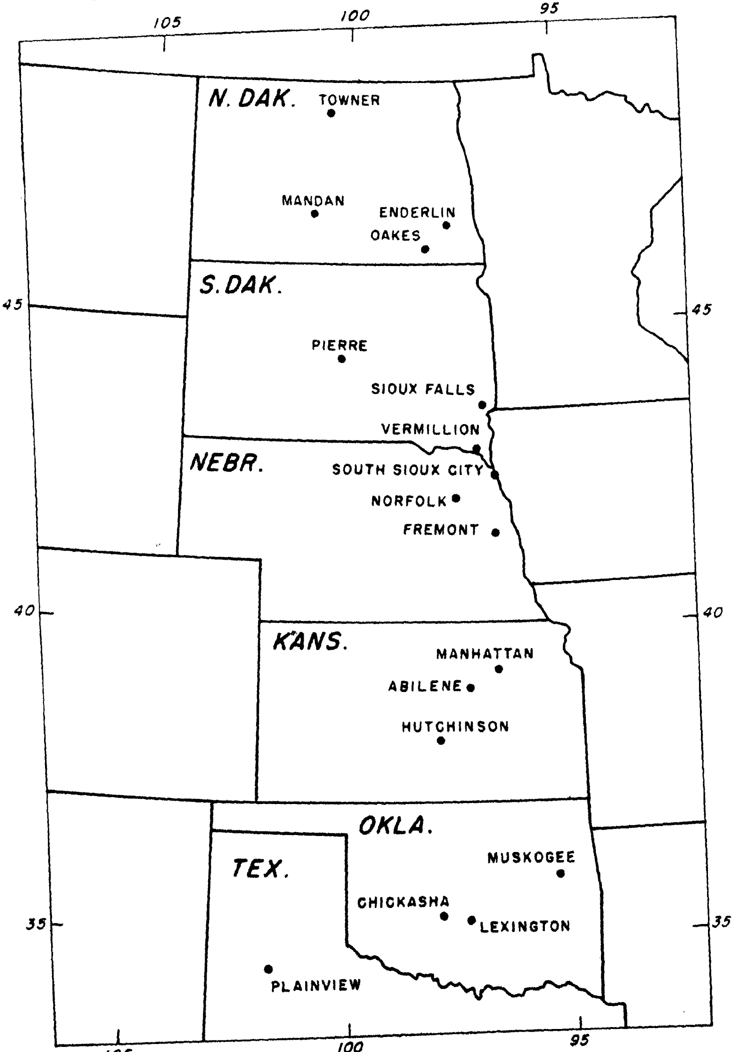


FIGURE 1.—Outline map, showing the distribution of nurseries of the Prairie States Forestry Project and Soil Conservation Service in the Great Plains region in 1940.

---

[5] Data from U. S. Weather Bureau, 30-year period, 1904-33.

tion was tall grass prairie and the soil developmental process was by calcification. Two of the nurseries, one at Mandan, N. Dak., and the other at Pierre, S. Dak., were within the Chestnut zonal soil group, which was also developed by calcification but on which the native vegetation was short grass prairie. Nurseries now or formerly located at Muskogee, Tecumseh, Oklahoma City, and Lexington, Okla.; Winfield, Kans.; and Fremont, Nebr., belong in the Prairie zonal soil group, which developed by calcification with some podzolization and originally had tall grass native vegetation (*18*).

The developmental process by calcification common to the soils of this region is characterized by such restricted leaching that the carbonates of calcium (lime) and magnesium accumulated somewhere in the lower or upper horizon of the soil (*8*). In reaction, therefore, the soils are characteristically neutral or slightly acid, frequently alkaline, but never strongly acid.

Climatic conditions and soil formations throughout the Great Plains region, therefore, are unusually uniform for such a great extent of latitude. The location of many of the broadleaf nurseries on sandy sites has further equalized natural soil differences. This uniformity tends to simplify the damping-off problem and makes it possible to compare losses directly between nurseries throughout the area.

CULTURAL PRACTICES IN PRODUCTION OF DECIDUOUS SEEDLINGS

Federal nurseries producing stock of broadleaf species in the Great Plains differ considerably from coniferous nurseries in this region. The deciduous seedlings are grown in drills or rows in open fields without benefit of artificial shade, frequent watering, or protection by seedbed frames (fig. 2). The general procedure is to sow the seed in drills,

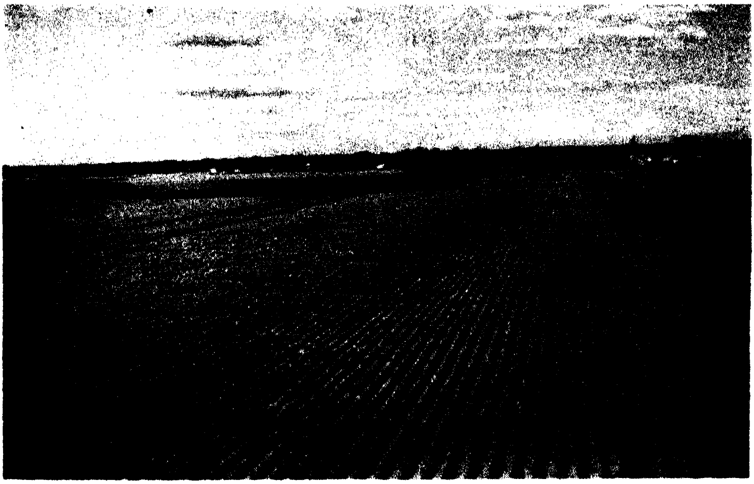


FIGURE 2.—Prairie States Forestry Project nursery at Fremont, Nebr., showing seedlings of broadleaf species grown in open, unprotected rows in the Great Plains region. Photograph from Forest Service, U. S. Department of Agriculture.

which are later ridged to prevent excessive drying of the soil in contact with the seed. As soon as the seeds begin to germinate the ridge is removed by hand raking. If germination is delayed by lack of precipitation, the sowings are irrigated by running a small stream of water between the rows. In a few nurseries water is supplied through elevated pipes, a method generally referred to as "overhead irrigation." After the seedlings emerge, they are irrigated from time to time, depending on the weather and the judgment of the nurserymen.

Density of sowing is mainly influenced by the viability of the seed and the probability that the seedlings will emerge and survive. The general practice is to sow the seed densely enough to obtain at least 16 seedlings per lineal foot of row. During the stage when the seedlings are most susceptible to damping-off they are on an average about one-half inch apart. Depth of sowing varies according to species.

The rows of seedlings are spaced from 21 to 30 inches apart, to permit machine cultivation and weeding in a manner similar to that used for truck crops. With the density and spacing described, there are from 100,000 to 250,000 seedlings grown per acre.

DAMPING-OFF OF SEEDLINGS OF BROADLEAF SPECIES

DESCRIPTION OF THE DISEASE

Two types of damping-off are common among broadleaf species in Great Plains nurseries, namely preemergence and postemergence damping-off. Preemergence damping-off, which results in the destruction of the seed or newly germinated seedling, prevents emergence and leaves vacant spots in the drills. This type of loss has frequently and erroneously been attributed to poor seed. Postemergence damping-off takes place after the seedlings emerge and may cause considerable loss until the plants are 3 to 4 weeks old. Sore shin,[6] which may be regarded as a phase of postemergence infection, stunts older seedlings but is not necessarily fatal and is quite common.

Damping-off caused by top infection has occurred only rarely in this region and is not regarded as an important problem during seasons of above-normal precipitation except in nurseries located on heavy soil. Late root rots caused by damping-off parasites are also of relatively little importance.

For broadleaf species, postemergence damping-off is usually atypical in that the infected seedlings do not become flaccid and fall prostrate on the soil. Instead, they commonly remain in an upright position, gradually wilt, break off, and finally are blown away. This type of loss has no doubt led to the prevailing opinion that postemergence damping-off of broadleaf species is rare. Only careful daily counts will prove otherwise, since such losses during 24 hours are seldom large but over a cumulative period of 10 days not uncommonly amount to 20 to 35 percent of the stand.

Postemergence damping-off of seedlings of broadleaf species is most severe when the seedlings are in the cotyledon stage. The heaviest losses occur at about the time the cotyledons cease to function and the true leaves are forming. As soon as the first true leaves are fully developed the critical damping-off period is past. At high soil temperatures the period of susceptibility may decrease, but losses are frequently greater, owing to increased activity of the fungus parasites.

---

[6] The term "sore shin" appears to have been used originally by Dr. George F. Atkinson in 1892 for describing a disease symptom of cotton in Alabama.

Diseased seedlings 2 weeks to 1 month old commonly show a reddish-brown lesion on the stem near the ground line (fig. 3). This condition, known as sore shin, is, as already mentioned, seldom fatal, but the growth of infected seedlings is reduced. These stunted plants occasionally do not develop to usable size in one season, particularly in northern nurseries.

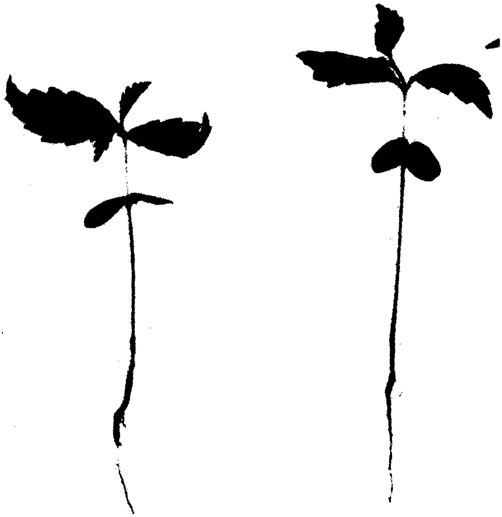


FIGURE 3.—Sore shin infection of American elm seedlings about 5 weeks old. Infection shows at base of seedlings. Note development of second pair of true leaves. These seedlings did not fall over and lie prostrate on the soil but continued to live.

SUSCEPTIBILITY OF SEEDLINGS IN THE FIELD

Field observations, together with greenhouse tests, indicated that not all deciduous seedlings are subject to damping-off. Table 1 gives the damping-off loss in percentage for several important broadleaf species at a number of different nurseries for the 3-year period 1936–38.

The following are the most important broadleaf species susceptible to damping-off in the field:

American elm	*Ulmus americana* L.
Siberian elm	*Ulmus pumila* L.
Chinese elm	*Ulmus parvifolia* Jacq.
Black locust	*Robinia pseudoacacia* L.
Russian mulberry	*Morus alba tatarica* (L.) Ser.
Desertwillow	*Chilopsis linearis* (Cav.) Sweet.
Russian-olive	*Elaeagnus angustifolia* L.

Table 1.—*Calculated damping-off losses in Great Plains nurseries, based on plot counts*

Year	Locality	Species	Plot counts[1]	Damping-off
			Number	*Percent*[2]
1936	Rayland, Tex	American elm	20	15
	do	Black locust	10	60
	Tecumseh, Okla	Siberian elm	10	60
	Noble, Okla	do	10	30
1937	Plainview, Tex	do	10	35
	do	Black locust	10	60
	Mangum, Okla	American elm	10	40
	do	Siberian elm	10	10
	Noble, Okla	do	12	20
	Manhattan, Kans	do	35	20
	Fremont, Nebr	do	120	35
	Enderlin, N. Dak	American elm	30	20
1938	Plainview, Tex	Siberian elm	25	10
	do	Desertwillow	25	10
	Noble, Okla	do	20	35
	Hutchinson, Kans	American elm	20	35
	do	Siberian elm	20	20
	Manhattan, Kans	American elm	30	15
	Norfolk, Nebr	Siberian elm	33	15

[1] Counts made in 1936 and 1937 were based on regularly distributed 1-foot quadrats, but in 1938 these were 3 feet long.

[2] Percentage of damping-off was determined by comparisons with zinc oxide treated seed; therefore the calculated losses are considerably lower than if based on stands growing under sterile conditions.

Damping-off losses were also heavy at times for other broadleaf species of less importance, such as Osage-orange (*Maclura pomifera* (Raf.) Schneid.), Siberian pea (*Caragana arborescens* Lam.), silverberry (*Shepherdia argentea* Nutt.), and dogwood (*Cornus* spp.).

A number of seedlings of broadleaf species showed high resistance to damping-off, and control measures have been unnecessary for such species. A list of the resistant species follows.

Green ash	*Fraxinus pennsylvanica* var. *lanceolata* (Borkh.) Sarg.
Northern catalpa	*Catalpa speciosa* Warder.
Common hackberry	*Celtis occidentalis* L.
Netleaf hackberry	*Celtis reticulata* Torr.
Common honeylocust	*Gleditsia triacanthos* L.
Bur oak	*Quercus macrocarpa* Michaux.
Common chokecherry	*Prunus virginiana* L.

Other, less important broadleaf species rarely seriously affected by damping-off fungi are: Kentucky coffeetree (*Gymnocladus dioicus* (L.) K. Koch), walnut (*Juglans* spp.), western soapberry (*Sapindus drummondii* Hook. and Arn.), and tree of heaven (*Ailanthus altissima* (Miller) Swingle).

DAMPING-OFF HAZARD IN NURSERY SOILS

In greenhouse tests, soil was taken from each nursery in 1935 and placed in 4-inch clay pots. Three pots, filled with the same unsterilized soil from 15 different nurseries, were each sown with 50 seeds of Siberian elm. One pot of each set was placed in a section of the greenhouse where air temperatures ranged from 55° to 70° F.; a second pot was held at temperatures ranging from 60° to 85°, and a third pot at temperatures of 65° to 100°. Moisture conditions were kept as nearly comparable as possible during the tests. Postemergence damping-off counts and isolations were made daily. Preemergence losses were verified by examinations at the end of the tests. Sterile sand checks, kept under similar conditions, made it possible to determine the losses on a percentage basis.

The nursery soils were grouped according to the severity of damping-off as shown in these tests. Since the tests were of limited extent, only an approximate arrangement was attempted. The results are summarized in table 2.

Hartley (*6*) and Jackson (*7*) found that incidence of damping-off of coniferous species is directly correlated with increase in pH values.

TABLE 2.—*Damping-off losses of Siberian elm grown in untreated nursery soils under greenhouse conditions*

SOILS WITH LIGHT DAMPING-OFF (LESS THAN 10 PERCENT)

Locality	Soil texture	Soil acidity	
		pH[1]	Depth
			Inches
Enid, Okla	Fine sandy loam	6.0	
Noble, Okla	Very fine sandy loam (containing lime).	8.8	

SOILS WITH MODERATE DAMPING-OFF (10 TO 30 PERCENT)

Locality	Soil texture	pH[1]	Depth
Enderlin, N. Dak	Fine sandy loam	7.2-7.5	
Valley City, N. Dak	Silty clay loam	6.9-7.5	
Baltic, S. Dak	do	7.0-7.5	
Arlington, Nebr	do	5.8 6.0	1 6
Fremont, Nebr	Silt loam	6.3 6.3	1 6
Abilene, Kans	do	6.0 6.4	1 6
Winfield, Kans	do	6.4	1
Oklahoma City, Okla	Silt clay loam	5.8-6.4	
Chillicothe, Tex	Fine sandy loam	7.2 7.0	1 6
Rayland, Tex	Very fine sandy loam	6.7	1

SOILS WITH HEAVY DAMPING-OFF (MORE THAN 50 PERCENT)

Locality	Soil texture	pH[1]	Depth
Brookings, S. Dak	Silty clay loam	7.8	
McPherson, Kans	do	6.0 6.0	1 1
Tecumseh, Okla	Silt to clay loam	6.0 6.5	1 6

[1] Colorimetric determinations.

In the tests conducted by the writer there does not appear to be a definite correlation between soil acidity and damping-off of Siberian elm. There was some indication in greenhouse tests, however, that damping-off losses of broadleaf species were least on sandy soils and heaviest on clay loam.

DAMPING-OFF FUNGI

ISOLATION TECHNIQUE

Diseased seedlings were collected daily from test pots in the greenhouse, and isolations of damping-off fungi were made. The technique used throughout this study was to remove the infected seedlings in toto and wash the roots in running water to remove the soil. The roots and root crown were then cut off with flame-sterilized scissors. A small section, ½ to 1 cm. long, was next cut from the infected part so as to include sound as well as obviously diseased tissue. These small sections were immersed in mercuric chloride (1:2,000) for 1 minute, rinsed thoroughly in sterile water, then placed between sterile filter paper for at least 5 minutes to remove excess water, and finally inserted into Petri dishes containing malt agar (pH 5.5). The cultures were incubated at 22° to 25° C. For later examination, transfers were made to malt agar in test-tube slants.

HOSTS AND LOCALITIES

In 1921 Hartley (*6*) listed several deciduous species reported to be infected with damping-off fungi. In a later paper Wright[7] summarized the literature up to 1937 and reported some additional broadleaf species affected with damping-off. Crandall[8] also added other hosts to the list. Since 1937 the writer has made many other isolations from diseased seedlings of broadleaf species. The results of these isolations are given by species and locality in table 3.

Pythium ultimum Trow was[9] the only species of *Pythium* commonly isolated from seedlings of broadleaf species showing symptoms of damping-off. No noticeable variations have been observed among the many *P. ultimum* isolates obtained in culture, either when coming from different hosts or from the same host growing in different soils.

TABLE 3.—*Isolations obtained from deciduous seedlings grown in Great Plains nursery soils*

Host	*Rhizoctonia*[1] *solani*	*Pythium*[2] *ultimum*	Locality
American elm (*Ulmus americana*).	......	×	Arlington, Nebr.
	×	......	Brookings, S. Dak.
	×	......	Fremont, Nebr.
	×	×	Lincoln, Nebr.
	×	......	Manhattan, Kans
	......	×	Muskogee, Okla.
	×	......	Oklahoma City, Okla.
	×	......	Rayland, Tex.
	×	......	Winfield, Kans.
Black locust (*Robinia pseudoacacia*).	×	×	Lincoln, Nebr.
	......	×	Manhattan, Kans.
	×	×	Rayland, Tex.
Siberian elm (*Ulmus pumila*).	......	×	Abilene, Kans.
	×	......	Arlington, Nebr.
	......	×	Baltic, S. Dak.
	×	......	Brookings, S. Dak.
	×	......	Chillicothe, Tex.
	×	......	Lexington, Okla.
	×	×	Lincoln, Nebr.
	×	......	Manhattan, Kans.
	×	×	Mandan, N. Dak.
	......	×	Noble, Okla.
	......	×	Oklahoma City, Okla.
	×	......	Rayland, Tex.
	×	......	Salina, Kans.
	×	×	Sioux Falls, S. Dak.
	×	×	Tecumseh, Okla.
	×	×	Winfield, Kans.
Desert willow (*Chilopsis linearis*).	×	×	Lincoln, Nebr.
	×	......	Rayland, Tex.
Green ash (*Fraxinus pennsylvanica* var. *lanceolata*).	×	......	Enid, Okla.
Siberian pea (*Caragana arborescens*).	×	......	Mandan, N. Dak.
Silver maple (*Acer saccharinum* L.)	×	......	Enid, Okla.
Northern catalpa (*Catalpa speciosa*).	......	×	Abilene, Kans.
	×	......	Arlington, Nebr.
	......	×	Winfield, Kans.

[1] All *R. solani* isolates were obtained from infected roots of young seedlings damping-off except that from green ash, which was suffering from top infection, and Siberian pea, 1 year old, showing late root rot.
[2] All *P. ultimum* isolates came from infected roots of young seedlings.

---

[7] WRIGHT, E. DECIDUOUS-SEEDLING DISEASES IN MIDWEST NURSERIES. U. S. Bur. Plant Indus. P Dis. Rptr. 21: 80–81. 1937. [Processed.]
[8] CRANDALL, B. S. RHIZOCTONIA ON TREE SEEDLINGS. U. S. Bur. Plant Indus. Plant Dis. Rptr. 21: 8 1937. [Processed.]
[9] Identification was verified through the courtesy of Dr. John T. Middleton, formerly of the Department of Botany, University of Missouri.

Variations were common among *Rhizoctonia solani* isolates obtained from different species, even when the hosts were grown in the same soil; these variations were most noticeable when the isolates were all grown on the same media and held under similar conditions. Macroscopic appearance has proved to be more dependable than microscopic measurements for distinguishing *R. solani* isolates. A similar conclusion was reached by Matz (*11*) and Briton-Jones (*2*).

All isolates obtained during this study are regarded as strains of *Rhizoctonia solani* Kühn. Cultural variations did not appear to be constant enough to justify separating the *Rhizoctonia* isolates into species as was done by Matz (*11*) and Simon-Thomas (*15*) (fig. 4). The *Corticium*, or perfect stage, was not observed in culture during the course of these investigations.

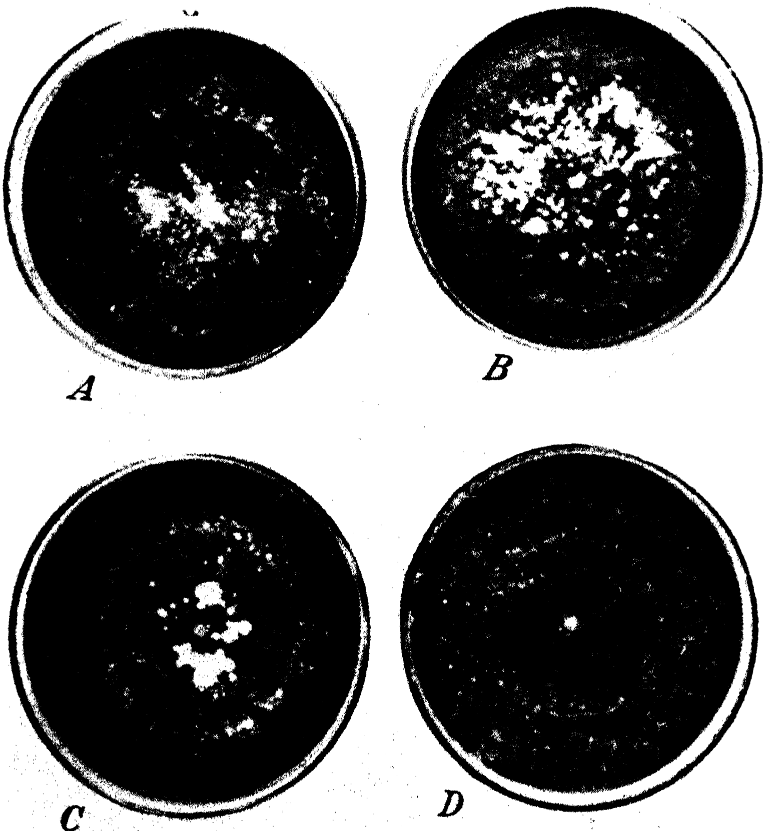


FIGURE 4.—Typical 2-week-old cultures of *Rhizoctonia solani* on malt agar. These isolations were obtained from seedlings of (*A*) American elm, (*B*) black locust, (*C*) Siberian elm, and (*D*) desertwillow, grown in the same Marshall silt loam soil. The cottony appearance of the sclerotia was characteristic of these *R. solani* isolates, but their abundance and distribution within the individual cultures were not always constant.

The isolations made during these studies indicated that American and Siberian elm seedlings are both commonly infected with *Rhizoctonia solani* and *Pythium ultimum*. Black locust appeared to be more frequently attacked by *P. ultimum*, and desertwillow by *R. solani*. Isolations for other broadleaf species were not numerous enough to warrant any conclusion as to which parasites were most prevalent, but *R. solani* and *P. ultimum* were most frequently isolated. *Fusarium* spp. also were frequently isolated from seedlings of broadleaf species that showed damping-off symptoms, and an occasional *Phytophthora* was obtained in culture. No attempt was made to identify the different isolates of *Fusarium*, since, judging by their prevalence, they were of secondary importance. All the *Fusarium* species obtained appeared to belong in the section *Elegans*.

It was not uncommon to obtain more than one organism in culture from the same diseased seedling. Frequently bacteria were present together with fungi, especially with *Pythium*. Occasionally *P. ultimum* and *Rhizoctonia solani* were isolated from the same seedling. During these studies the sclerotia of *R. solani*, such as are commonly found on potato tubers, were never observed on any of the broadleaf species. This may be due to the fact that after infection the seedlings soon decay.

PATHOGENICITY TESTS THROUGH SOIL INOCULATION

Matsumoto (*10*), Richter (*13*), and Simon-Thomas (*15*) have concluded that *Rhizoctonia solani* is more virulent on the host from which it was originally isolated than it is on a different host species. Gratz (*5*) and Wellman (*19*) found that *R. solani* from potatoes did not cause wire stem of cabbage, while Lauritzen (*9*) and Tervet (*16*) reported that potato isolates of this fungus were nonpathogenic to turnip and flax. The investigations of Sanford (*14*) and Garrett (*4*) showed that soil-inoculation tests are influenced not only by the character of the inoculum but also by soil sterilization. Hartley (*6*) had found earlier that inoculations with *Pythium debaryanum* Hesse[10] on unheated soils were much less destructive than on heated soils, and Sanford (*14*) found the opposite to be true of *R. solani*.

In the pathogenicity tests reported here an attempt was made to take these various factors into consideration, and a number of tests were made in both sterilized and unsterilized soil. In making soil inoculations for pathogenicity tests, about 1/10 of a 90-mm. malt-agar Petri-dish culture was worked into the surface soil of each pot. Inoculations were made in both sterilized and unsterilized soil. Several days after inoculation, the seeds of broadleaf species were sown.

Early in these tests isolates of *Rhizoctonia* from American and Siberian elms (fig. 4) were used in a small series of cross inoculations in steam-sterilized soil. One-month-old cultures containing well-developed sclerotia were used. Eight 4-inch clay pots for each series were inoculated, and 3 days after inoculation the seeds were sown. Seeds were sown in the same number of pots containing sterilized, uninoculated soil, to determine seed viability as a basis for estimating preemergence losses. The results of these tests are presented in table 4.

---

[10] Hartley later expressed the opinion that the identification of the damping-off *Pythium* as *P. debaryanum* was probably incorrect and that he was working with *P. ultimum*.

TABLE 4.—*Pathogenicity tests of 2 Rhizoctonia solani strains introduced into sterilized loam*

[Air temperature, 60°-80°F.; duration of tests, 30 days]

AMERICAN ELM RHIZOCTONIA

Crop[1]	Total emergence		Preemergence loss based on checks	Postemergence loss based on stand in inoculated pots	Final stand based on checks
	Check	Inoculated			
	Number	*Number*	*Percent*	*Percent*	*Percent*
Ponderosa pine	320	0	100		
Siberian elm	268	0	100		
Wheat (winter)	392	321	18	97	2
Alfalfa (Grimm)	356	128	64	88	5
Corn (Hogue Yellow Dent)	194	194	0	0	100

SIBERIAN ELM RHIZOCTONIA

Crop[1]	Check	Inoculated	Preemergence loss based on checks	Postemergence loss based on stand in inoculated pots	Final stand based on checks
Ponderosa pine	320	135	58	10	38
Siberian elm	268	43	84	40	9
Wheat (winter)	392	314	20	57	49
Alfalfa (Grimm)	356	331	7	0	93
Corn (Hogue Yellow Dent)	194	183	6	0	94

[1] 400 seeds were used for all species except corn, for which 200 seeds were used. 8 pots were used for each test. Inoculations were made 3 days before sowing.

It is apparent from these cross-inoculations that the *Rhizoctonia solani* strains from both American and Siberian elms are pathogenic to a number of crops. However, the *R. solani* isolate from American elm proved somewhat more virulent than that from Siberian elm. This was particularly evident on ponderosa pine and Siberian elm. These results show that *R. solani* can cause heavy preemergence losses. The two *R. solani* strains tested, however, caused practically no preemergence loss for corn and very little for wheat. Postemergence infection of these two crops was nevertheless heavy. The wheat exhibited true postemergence damping-off symptoms by falling prostrate to the soil, but the corn showed only brownish infection spots, or sore shin, near the ground line. The infection spots were overgrown by the end of the tests, and there was no damping-off.

In a larger series of soil inoculations the pathogenicity of both *Pythium ultimum* and several strains of *Rhizoctonia solani* was tested on four broadleaf hosts. Marshall silt loam contained in 4-inch clay pots was inoculated with a week-old malt-agar culture. Twenty pots of steam-sterilized and unsterilized soil constituted each inoculation series, and the same number of pots containing uninoculated sterilized and unsterilized soil were set up as checks. All pots were sown with 100 seeds. The percentage of preemergence and postemergence damping-off occurring in these tests is given in table 5.

The general trend of the preemergence losses after inoculation with *Rhizoctonia solani* was greater in unsterilized than in sterilized soil, whereas the occurrence of postemergence damping-off was greater in sterilized soil. *Pythium ultimum* caused greater preemergence losses, except for desertwillow, in sterilized soil, in which, accordingly, less postemergence damping-off occurred. These tests did not show that the *R. solani* strains tested were most virulent to the host from which

TABLE 5.—*Effect of cross-inoculations in soil with Rhizoctonia solani and Pythium ultimum originally isolated from seedlings of broadleaf species growing in the same soil*

[5 pots of 100 seeds each]

STERILIZED SOIL[1]

Fungus inoculated into soil, and host source	American elm				Siberian elm				Black locust				Desertwillow			
	Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss		Preemergence loss		Postemergence loss	
	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Rhizoctonia solani **from—**																
American elm	39	±4.9	37	±5.4	57	±3.8	22	±4.8	47	±4.9	28	±2.0	54	±6.2	24	±1.7
Siberian elm	25	±6.8	48	±5.9	63	±2.8	17	±2.2	52	±3.6	27	±1.4	53	±6.2	28	±2.2
Black locust	25	±2.7	21	±5.9	39	±1.7	11	±5.5	28	±3.5	21	±2.0	41	±2.6	11	±.5
Desertwillow	65	±2.3	10	±3.3	64	±2.3	11	±2.8	62	±2.7	13	±3.3	71	±.21	4	±2.2
Pythium ultimum **from—**																
Siberian elm	40	±8.1	21	±5.2	40	±8.3	19	±2.8	38	±3.1	12	±2.0	68	±4.3	4	±2.0
Uninoculated soil	26	±9.9	29	±3.1	17	±6.2	18	±5.1	4	±2.3	18	±5.4	38	±1.2	18	±4.3
UNSTERILIZED SOIL																
Rhizoctonia solani **from—**																
American elm	64	±5.9	9	±2.6	74	±2.1	5	±0.18	69	±1.6	10	±3.3	60	±2.4	15	±2.0
Siberian elm	75	±1.6	5	±2.2	81	±.1	1	±.70	79	±1.9	5	±2.0	70	±2.8	11	±1.7
Black locust	38	±5.8	10	±2.0	43	±2.9	7	±1.4	34	±3.9	14	±[illegible].2	29	±7.5	12	±3.3
Desertwillow	64	±3.5	7	±5.0	66	±3.9	10	±2.2	53	±4.3	18	±1.7	55	±3.9	20	±3.7
Pythium ultimum **from—**																
Siberian elm	62	±2.9	9	±1.4	78	±.1	2	±.30	36	±4.2	9	±3.4	56	±3.5	11	±4.4
Uninoculated soil	63	±2.5	7	±1.4	77	±2.2	1	±.60	29	±4.1	13	±3.8	54	±6.4	18	±4.1

---

[1] Marshall silt loam soil steam-sterilized 1 hour at 5 pounds' pressure.

each was originally isolated. The question naturally arises as to why these results do not agree with the findings of Matsumoto (*10*), Richter (*13*), and Simon-Thomas (*15*). There are several possible explanations for this. The most probable is that the seedlings of the broadleaf species tested were all quite similar and did not show marked anatomical differences such as are displayed by agricultural crops, e. g., potatoes and sugar beets. It may also be significant that the isolates comprising the inocula were all obtained from diseased seedlings grown in the same soil. In the literature this point has not always been made clear, since the emphasis has been placed on the host rather than on both host and soil type. When these points are considered, it is more apparent why these tests do not show clear-cut variations in pathogenicity between isolates.

Of the broadleaf species used, black locust showed the greatest resistance to damping-off by both *Rhizoctonia solani* and *Pythium ultimum*. This reaction applied to the *R. solani* strain from black locust as well as to the isolates from the other hosts. It is known that these are atypical reactions for black locust, and it is probable that this apparent resistance was actually a partial escape from infection. Because of prompt seed germination and vigorous growth, the black locust seedlings appear to have passed the stage of greatest susceptibility before the soil inoculations became completely effective.

When black locust seedlings attacked by *Rhizoctonia solani* were examined soon after infection, it was found that discoloration and decay usually occurred first in the region of the root crown and that the tip of the root was destroyed last. Davis (*3*) described similar symptoms for longleaf pine seedlings infected with *Rhizoctonia solani*. Typical *Pythium ultimum* infection, however, early caused a decay of the tip of the root, and the root crown was not attacked until later. Hence the progress of infection for these two parasites was commonly directly opposite. Figure 5 shows these two types of

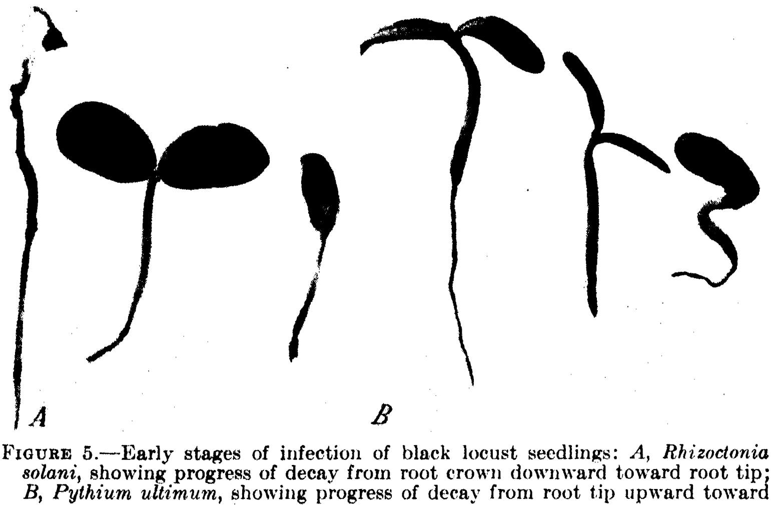


FIGURE 5.—Early stages of infection of black locust seedlings: *A*, *Rhizoctonia solani*, showing progress of decay from root crown downward toward root tip; *B*, *Pythium ultimum*, showing progress of decay from root tip upward toward root crown.

infection on black locust seedlings. Since the sterilized, uninoculated soil checks in the previous test also suffered from damping-off, a brief summary of the isolations obtained from diseased seedlings is presented in table 6.

TABLE 6.—*Summary of isolations obtained from damped-off seedlings in test presented in table 5*

Soil treatment and fungus used in inoculation	Isolate					
	R. solani		*P. ultimum*		Unclassified[1]	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Sterilized soil:						
Rhizoctonia solani	107	68	5	3	46	29
Pythium ultimum	0		23	60	16	40
Checks	0		19	100	0	
Unsterilized soil:						
Rhizoctonia solani	68	58	18	15	32	27
Pythium ultimum	0		16	70	7	30
Checks	0		11	44	14	56

[1] Bacteria, other fungi, and blanks.

The isolations obtained from damped-off seedlings showed that steam-sterilized, uninoculated soil was contaminated with *Pythium ultimum*. Contamination probably came from adjacent inoculated pots through splashing of water. There were also some *P. ultimum* contaminations in the sterilized soil inoculated with *Rhizoctonia solani*. There were, however, no contaminations of *R. solani* in any of the series not inoculated with this fungus.

Regardless of the lack of variation in virulence between isolates or accidental contaminations, these tests show that *Pythium ultimum* and the *Rhizoctonia solani* strains tested are definitely pathogenic to young seedlings of American and Siberian elms, black locust, and desertwillow, as well as to several agronomic hosts. This comparatively wide range of pathogenicity further justifies designating these isolates of *Rhizoctonia* as strains rather than species. This is in accord with the view of Peltier (*12*), Tilford (*17*), and Wiant (*20*).

Preliminary tests with seedlings of these same four broadleaf species were made by inoculating sterilized and unsterilized soil with *Fusarium* sp. The method of inoculation used was similar to that employed for *Rhizoctonia* and *Pythium*. The results of these tests indicated that *Fusarium* is capable of causing fairly heavy preemergence losses in sterilized inoculated soil but very little in unsterilized inoculated soil. However, postemergence damping-off in sterilized soil was very light, whereas in unsterilized soil such losses were fairly heavy. *Fusarium*, therefore, may cause postemergence damping-off in the field when climatic conditions are favorable and in such instances may influence control measures.

A series of soil inoculations was also made to determine the effect of soil sterilization on the pathogenicity of *Pythium ultimum* and *Rhizoctonia solani*.

Thirty 7-inch glazed-earthenware crocks were filled with Marshall silt loam (pH 5.5–6.0) collected at Lincoln, Nebr. Fifteen of the crocks were sterilized for 3 hours at 20 pounds' pressure. The sterilized soil in 5 crocks was inoculated with *Pythium ultimum*, and the sterilized soil in 5 other crocks was inoculated with *Rhizoctonia solani*, leaving 5 crocks of soil uninoculated as checks. Two-week-old inoculum was used. Immediately after inoculation, each of the 30 crocks was sown with 100 seeds of Siberian elm. The entire lot was then placed in cases with air temperature held at 75° F. Five days later the soil first inoculated with *P. ultimum* was inoculated with *R. solani*, and that first inoculated with *R. solani* was inoculated with *P. ultimum*. Damping-off counts and isolations were made periodically. The results of this series of tests are presented in table 7.

TABLE 7.—*Results of sowing Siberian elm seed in soil inoculated with both Pythium ultimum and Rhizoctonia solani* [1]

Soil treatment	Total emergence	Preemergence loss based on noninoculated pots	Postemergence damping-off based on stand	Final stand based on noninoculated pots
Pythium-inoculated soil plus *R. solani* 5 days later:	*Number*	*Percent*	*Percent*	*Percent*
Sterilized soil	18	95	61	2
Unsterilized soil	150	36	81	10
Rhizoctonia-inoculated soil plus *P. ultimum* 5 days later:				
Sterilized soil	178	49	80	11
Unsterilized soil	76	68	47	18
Uninoculated soil (check):				
Sterilized soil	348	0	0	100
Unsterilized soil	236	32	28	48

[1] 5 crocks of sterilized and 5 of unsterilized soil were used in each test.

Table 7 shows that the soil first inoculated with *Pythium ultimum* reacted in a manner directly opposite to that of the soil first inoculated with *Rhizoctonia solani*. For example, the greater preemergence loss for the *P. ultimum* series was in the sterilized inoculated soil, whereas that for the *R. solani* series was in the unsterilized inoculated soil. Conversely, the heaviest postemergence damping-off occurred on the unsterilized soil when it was first inoculated with *P. ultimum*, and the heaviest loss occurred on the sterilized soil when it was first inoculated with *R. solani*. This apparent contradiction is clarified by the isolation data presented in table 8.

TABLE 8.—*Isolates obtained from Siberian elm seedlings showing postemergence damping-off symptoms in test presented in table 7*

Isolate	Isolations from—									
	Pythium-inoculated soil plus *R. solani* 5 days later				*Rhizoctonia*-inoculated soil plus *P. ultimum* 5 days later				Uninoculated unsterilized soil	
	Sterilized soil		Unsterilized soil		Sterilized soil		Unsterilized soil			
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Pythium ultimum	3	60	3	7	2	4	0		5	28
Rhizoctonia solani	1	20	27	59	42	82	8	62	1	5
Trichoderma sp	1	20	0		1	2	0		0	
Fusarium sp	0		5	11	1	2	1	8	8	45
Phycomycetes	0		2	4	3	6	0		1	5
Alternaria sp	0		0		2	4	0		1	5
Aspergillus sp	0		1	2	0		1	8	0	
Penicillium sp	0		0		0		1	8	1	5
Bacteria	0		6	13	0		2	15	1	5
Unclassified	0		2	4	0		0		0	
Total	5	100	46	100	51	100	13	101	18	98

All these tests show that both *Pythium ultimum* and *Rhizoctonia solani* are definitely parasitic on broadleaf seedlings. Pathogenicity can be obtained in either sterilized or unsterilized soil, depending upon whether the tests are for preemergence or postemergence damping-off. Preemergence losses caused by *P. ultimum* apparently can be better demonstrated for broadleaf species by inoculation in sterilized rather than unsterilized soil, but *R. solani* inoculations were more effective in unsterilized soil. On the other hand, for producing postemergence damping-off, these tests showed that the percentage loss was greater when *P. ultimum* was inoculated into unsterilized soil and *R. solani* into sterilized soil.

DISCUSSION

The information presented in this paper is believed to be fundamental to a complete understanding of the damping-off problem as it affects seedlings of broadleaf species in Great Plains nurseries. Because of the uniformity in climatic conditions and soil formations through the Great Plains region, control measures that are successful in Texas are likely to be equally so in North Dakota. In some respects the problem of controlling damping-off of broadleaf species is similar to that of its control in other crops because the principal parasites in both cases are *Pythium ultimum* and *Rhizoctonia solani* and on occasion *Fusarium* spp. Since the seedlings are commonly grown in open drills and not in protected seedbeds, successfull large-scale control measures will necessarily be quite different from those used in coniferous nurseries.

Only about half of the broadleaf species studied proved to be susceptible to damping-off; therefore the control problem at once becomes considerably simplified. Since some soils of the region showed heavier damping-off potentialities than others, only those broadleaf species resistant to damping-off should be grown on unfavorable sites. For susceptible broadleaf species, control methods should take into consideration the fact that both *Pythium ultimum* and *Rhizoctonia solani* are pathogenic and that *Fusarium* is apparently also capable of causing postemergence losses when temperature conditions are favorable. Treatments that inhibit one of these parasites may encourage the development of another. Moreover, there does not appear to be a marked difference in virulence between *Rhizoctonia* strains; hence, broadleaf species susceptible to damping-off should not be grown in the area occupied during the preceding season by other susceptible species. If these findings are taken into consideration, control measures should be somewhat simplified and more effective.

SUMMARY

Soil developmental processes and climatic conditions in the Great Plains are unusually uniform for a region extending through so many degrees of latitude.

Approximately 50 percent of the broadleaf species grown are susceptible to damping-off, four of the most important being the American elm, Siberian elm, black locust, and desertwillow.

For susceptible species, damping-off mainly assumes two forms. When the seed or new radicle is destroyed, the losses are classified as

preemergence damping-off. Postemergence damping-off results from infection of seedlings from a few days to 3 weeks old, and the diseased plants may either fall prostrate on the soil or, more commonly, remain erect, dry up, and break off. Sore shin is the result of stem infection at about ground level of older plants. Root rots and top infection of young seedlings are unimportant in this region.

The principal damping-off fungi have been identified as *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow; *Fusarium* spp. may also be of occasional importance. *R. solani* isolates from different broadleaf species are regarded as strains, since cultural characteristics were not constant enough, in the opinion of the writer, to justify the establishment of species. Of the broadleaf species tested, black locust appeared to be the most susceptible to *P. ultimum* and desertwillow the most susceptible to *R. solani*.

Pathogenicity tests have proved that *Pythium ultimum* and several strains of *Rhizoctonia solani* are definitely pathogenic to a number of broadleaf species and also to several kinds of agronomic plants. *R. solani* inoculations appeared to be more successful in unsterilized soil than in sterilized soil, whereas the opposite was true for *P. ultimum*. The comparatively wide range of pathogenicity of *R. solani* isolates further justified designating them as strains rather than species. Preliminary tests indicated that *Fusarium* spp. can cause fairly heavy postemergence damping-off in unsterilized, inoculated soil but very little preemergence loss except in sterilized, inoculated soil.

The relation of these studies to the control of damping-off of broadleaf species is discussed.

LITERATURE CITED

(1) Boyce, J. S.
1938. FOREST PATHOLOGY. 600 pp., illus. New York and London.
(2) Briton-Jones, H. R.
1924. STRAINS OF RHIZOCTONIA SOLANI KÜHN (CORTICIUM VAGUM BERK. AND CURT.). Brit. Mycol. Soc. Trans. 9: 200–210, illus.
(3) Davis, W. C.
1941. DAMPING-OFF OF LONGLEAF PINE. Phytopathology 31: 1011–1016.
(4) Garrett, S. D.
1937. THE SOIL-BORNE FUNGUS DISEASES OF FIELD AND PLANTATION CROPS: A REVIEW OF EXISTING CONTROL METHODS. Empire Jour. Expt. Agr. 5: 189–196.
(5) Gratz, L. O.
1925. WIRE STEM OF CABBAGE. N. Y. (Cornell) Agr. Expt. Sta. Mem. 85, 60 pp., illus.
(6) Hartley, C.
1921. DAMPING-OFF IN FOREST NURSERIES. U. S. Dept. Agr. Bul. 934, 99 pp., illus.
(7) Jackson, L. W. R.
1940. EFFECTS OF H-ION AND AL-ION CONCENTRATIONS ON DAMPING-OFF OF CONIFERS AND CERTAIN CAUSATIVE FUNGI. Phytopathology 30: 563–579, illus.
(8) Kellogg, C. E.
1936. DEVELOPMENT AND SIGNIFICANCE OF THE GREAT SOIL GROUPS OF THE UNITED STATES. U. S. Dept. Agr. Misc. Pub. 229, 40 pp., illus.
(9) Lauritzen, J. I.
1929. RHIZOCTONIA ROT OF TURNIPS IN STORAGE. Jour. Agr. Res. 38: 93–108, illus.
(10) Matsumoto, T.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XII. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA SOLANI KÜHN. Mo. Bot. Gard. Ann. 8: 1–62, illus.

(11) Matz, J.
1921. THE RHIZOCTONIAS OF PORTO RICO. Porto Rico Dept. Agr. Jour. 5(1): 5-31, illus.
(12) Peltier, G. L.
1916. PARASITIC RHIZOCTONIAS IN AMERICA. Ill. Agr. Expt. Sta. Bul. 189: 283-390, illus.
(13) Richter, H.
1936. FUSSKRANKHEIT UND WURZELFÄULE DER LUPINE (ERREGER: RHIZOCTONIA SOLANI K.). Zentbl. f. Bakt., Abt. (II), 94: 127-133, illus.
(14) Sanford, G. B.
1939. RESEARCH ON CERTAIN SOIL-BORNE DISEASES AS AFFECTED BY OTHER MICRO-ORGANISMS. Sci. Agr. 19: 609-615.
(15) Simon-Thomas, K.
1925. ONDERZOEKINGEN OVER RHIZOCTONIA. 97 pp., illus. Utrecht.
(16) Tervet, I. W.
1937. AN EXPERIMENTAL STUDY OF SOME FUNGI INJURIOUS TO SEEDLING FLAX. Phytopathology 27: 531-546, illus.
(17) Tilford, P. E.
1930. A RHIZOCTONIA DISEASE OF SWEET ALYSSUM. Phytopathology 20: 587-590, illus.
(18) Weaver, J. E., and Clements, F. E.
1938. PLANT ECOLOGY. Ed. 2, 601 pp., illus. New York and London.
(19) Wellman, F. L.
1932. RHIZOCTONIA BOTTOM ROT AND HEAD ROT OF CABBAGE. Jour. Agr. Res. 45: 461-469, illus.
(20) Wiant, J. S.
1929. THE RHIZOCTONIA DAMPING-OFF OF CONIFERS, AND ITS CONTROL BY CHEMICAL TREATMENT OF THE SOIL. N. Y. (Cornell) Agr. Expt. Sta. Mem. 124, 64 pp., illus.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., AUGUST 1, 1944 No. 3

ANATOMICAL AND CYTOLOGICAL STUDIES ON BEET MOSAIC[1]

By KATHERINE ESAU

Assistant botanist, California Agricultural Experiment Station

INTRODUCTION

The anatomy of the sugar beet (*Beta vulgaris* L.) affected with mosaic has been investigated by several workers, but some results are contradictory. Robbins (*46*)[2] described necrosis of the vascular tissues and of the adjacent parenchyma in mosaic beets, and Roland (*47*) saw gummosis in the phloem tissue of beets affected by "jaunisse," which he regarded as similar to beet mosaic. Böning (*7*), Schaffnit and Weber (*48*), and Sirotina (*54*), on the other hand, reported no phloem necrosis in mosaic beets. Schaffnit and Weber (*48*) found foreign bodies in the phloem of mosaic sugar beets, but Sirotina (*54*) noted such bodies in healthy plants also. According to the modern concept, a mosaic virus is not limited to the phloem and produces few or no abnormalities in this tissue (*5*, *21*); it therefore appeared pertinent to reexamine the phloem of beets affected with mosaic. The first part of the present paper compares the phloem of healthy and mosaic-diseased sugar beets.

The second part deals with the structure of the mesophyll in mosaic beet leaves, particularly with plastid abnormalities. According to many workers (*7–15*, *25*, *32*, *34*, *42*, *53*, *57*), the yellow areas in leaves affected with mosaics are hypoplastic and contain small chloroplasts reduced in numbers as compared with healthy mesophyll or with the dark-green areas of mosaic leaves. Some workers strongly emphasize that underdevelopment is the only abnormality of the yellow areas, that cell contents are not destroyed, and that the affected cells continue to develop until they become more nearly like those of the green areas (*7*, *8*, *10*, *11*, *12*, *13*). Although recognizing hypoplasia as the principal disease symptom in the yellow areas, other workers record also some injury to the contents, particularly the plastids (*9*, *14*, *25*, *32*, *44*). The present study is concerned especially with the question whether underdevelopment alone explains the peculiarities of the yellow areas in mosaic leaves, or whether destructive changes also occur in them.

---

[1] Received for publication March 25, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 114.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 3
August 1, 1944
Key No Calif.-136

MATERIALS AND METHODS

The mosaic-diseased sugar-beet material was obtained from artificially inoculated greenhouse plants grown at Riverside, Calif.,[3] and from naturally inoculated plants in fields of the Sacramento Valley. Healthy leaves were collected from greenhouse plants and in fields that showed no mosaic in the years when this disease was not prevalent. Symptom-free plants in severely affected fields were also sampled. The material was examined partly in the fresh state in freehand sections mounted in water, partly after treatment by a common paraffin method. Tap and distilled water were tried for the fresh sections, but the former was selected as the less injurious medium. Several killing solutions were employed in the paraffin method, mainly formalin-aceto-alcohol mixtures, Randolph's modified Navashin fluid (*35, p. 45*), and a bichromate-chromic-osmic acid solution according to Champy (*40, p. 37*). The paraffin sections were cut 7 to 10 microns thick and were stained with Heidenhain's haematoxylin and safranine as used previously by the writer (*22*)[4]. This staining method gives a good wall differentiation and also shows cytoplasmic details. The stain may be variously balanced so as to have either the haematoxylin or the safranine predominating in the cytoplasmic structures. In detail, the schedule is as follows:

(1) Place slides, with paraffin removed, in distilled water.

(2) Change distilled water 5 times during 10 minutes.

(3) Mordant in 4-percent aqueous ferric ammonium sulphate for 10 minutes.

(4) Transfer to distilled water, and change the latter 4 or 5 times during 10 to 20 minutes.

(5) Transfer into a weak solution of haematoxylin prepared by putting 10 drops of stock solution (2.5 gm. of haematoxylin crystals in 100 cc. of 95-percent ethyl alcohol) into a Coplin staining dish with tap water or with distilled water made slightly alkaline with sodium bicarbonate. Stain until the walls appear bluish. The time varies with the kind of material, the age of the organ, and the fixing solution used. From 10 to 20 minutes was required in the present investigation.

(6) Rinse in tap water.

(7) Stain 6 to 24 hours in a dilute safranine solution prepared by adding 5 to 6 drops of stock solution (1 gm. safranine O in 100 cc. of 50-percent alcohol) to a Coplin dish with tap water.

(8) Destain in 50-percent alcohol, and carry through higher alcohols to xylene. Treat each slide individually.

(9) Mount in balsam.

The larger photographs were taken on Isopan film, the smaller on Finopan film. Wratten filters B-58 (green) and 44 (minus red) were used in photography. The drawings were prepared with the aid of a camera lucida. All photographs were made from paraffin material, the drawings mostly from freehand sections.

EXTERNAL SYMPTOMS OF BEET MOSAIC

As several workers have indicated (*7, 43, 46, 54, 58*), the yellow-green mottling of the mosaic beet leaves shows various patterns and different degrees of paleness and distinctness of the yellow areas. Masking of symptoms also occurs in this disease. Figure 1 shows a healthy leaf and a mottled mosaic leaf of greenhouse plants from Riverside, and figure 2 illustrates the common patterns of mottling found on mosaic beets in a field near Davis. Leaf *A* in figure 2 has the diffuse type of mottling characteristic of older leaves. In leaf *B* the yellow areas are comparatively large and sharply delimited. This pattern somewhat resembles that of the mosaic leaf in figure 1. The contrast between the yellow and green areas is particularly sharp in leaf *C* (fig. 2). In leaf *D* the yellow areas have the shape of small roundish spots.

---

[3] This material was supplied by Dr. C. W. Bennett, Division of Sugar Plant Investigations, U. S. Department of Agriculture.

[4] This method of staining was originally recommended by Dr. R. H. Wetmore, of Harvard University.

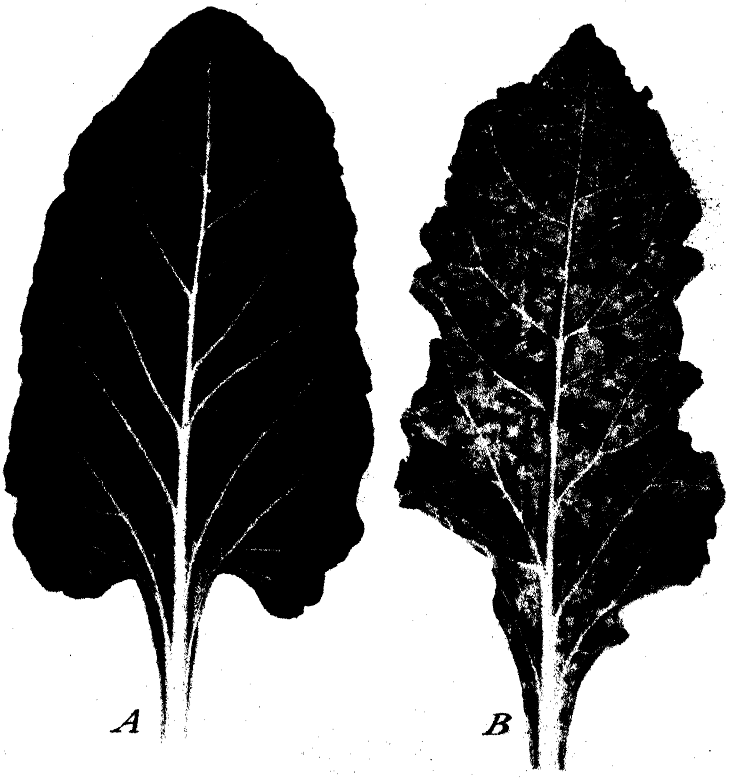


FIGURE 1.—*A*, Healthy leaf and *B*, mosaic-diseased leaf from sugar-beet plants grown in a greenhouse at Riverside, Calif. The diseased plant was artificially inoculated with the mosaic virus. (Photograph furnished by Dr. C. W. Bennett.) ×1.

ANATOMY OF THE PHLOEM OF MOSAIC LEAVES

The present writer (*18*, *19*) has already described the development and structure of the phloem in healthy sugar-beet leaves. Briefly, the functioning phloem as seen in a transverse section of a bundle (pl. 1, *A*) is a small-celled tissue composed of sieve tubes, very small companion cells, and phloem parenchyma cells. These last are the largest in the tissue. The older part of the primary phloem which ceased to function constitutes the bundle cap (pl. 1, *A*, left). Within it the remnants of crushed sieve tubes and companion cells seem like deeply stained wall thickenings. The intact cells of the cap are the much-elongated phloem cells, which develop rather thick cellulose walls after the conducting elements of the phloem are obliterated.

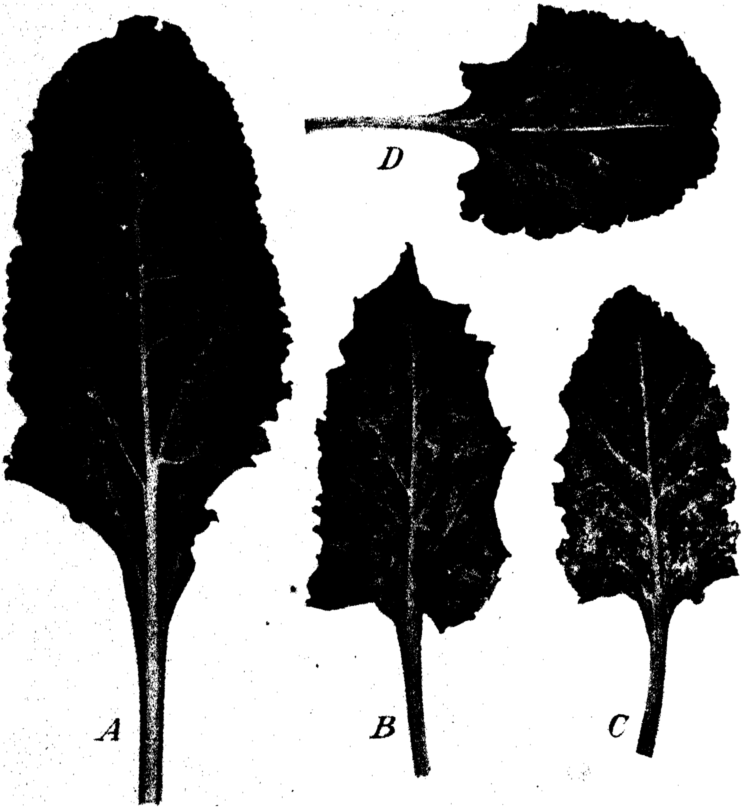
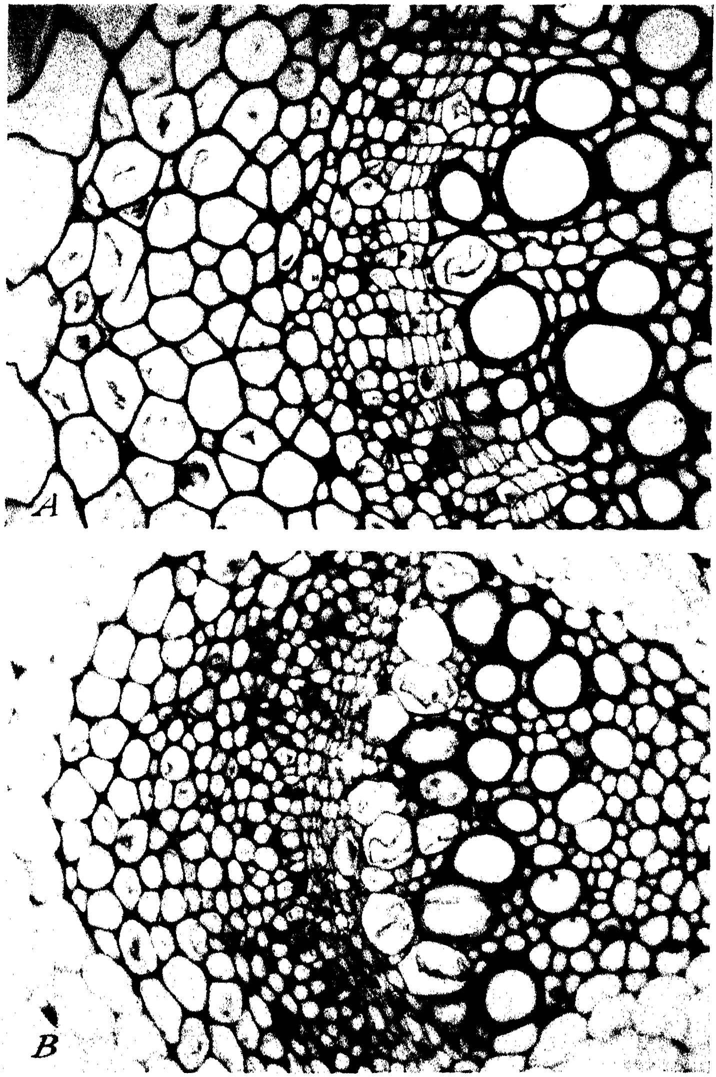


FIGURE 2.—Leaves from mosaic-diseased sugar-beet plants from a naturally infected commercial planting near Davis, Calif.: *A*, Diffuse type of mottling characteristic of older leaves; *B*, yellow areas comparatively large and sharply delimited; *C*, contrast between yellow and green areas particularly sharp; *D*, yellow areas appearing as small roundish spots. × ½.

Younger and older leaves from many severely affected plants grown in greenhouse and outdoors were examined for phloem structure. No abnormalities were observed in the vascular tissues, when the leaves



Transverse sections of vascular bundles from healthy (*A*) and mosaic-diseased (*B*) beet leaves. × 290.

showed no necrosis externally. A vascular bundle of a young, severely mottled leaf from a field-grown plant appears in plate 1, *B*. This bundle is younger than that in plate 1, *A*. It shows normal phloem and an immature bundle cap in which the different stages in obliteration of the sieve tubes and companion cells are conspicuous. The phloem of sugar-beet leaves affected with curly top strikingly contrasts with that of the mosaic leaves. Curly top induces hypertrophy and hyperplasia in the phloem of affected plants. The tissue resulting from these growth abnormalities sharply deviates from the normal phloem (*18*, *20*). Eventually it collapses, and a gumlike substance accumulates among the dead cells.

Certain mosaic leaves used in the present study showed necrosis of various tissues. Thus necrosis of phloem, xylem, mesophyll, and epidermis was observed in the local lesions that sometimes appeared at the place of inoculation and in yellow areas along the veins through which the virus passed from the place of inoculation before systemic infection occurred. The necrosis was a collapse of cells that appeared to have developed normally, accompanied by an accumulation of deeply staining material. Judging from these instances of phloem necrosis, this abnormality may occur under certain conditions in mosaic beet, but is not a constant or specific symptom and does not indicate a close relation of the mosaic virus to the phloem. In contrast, phloem degeneration in curly top diseased plants is a constant primary symptom and is one indication of the close association between the virus and the phloem of the host. The other pathologic changes in mosaic and curly top beets point in the same direction. The stunting of plants and the malformations on leaves in curly top appear to be secondary symptoms that can be explained as resulting from disturbances in the phloem tissue (*18*, *20*). The mesophyll abnormalities in mosaic beets, on the other hand, can be well interpreted as primary symptoms induced by the virus in this tissue.

It is difficult to explain the necrosis of entire mesophyll cells and even of the vascular tissues observed in the mosaic material just described while such necrosis is absent in leaves that develop after the systemic infection has become established. Conceivably, at the place of inoculation and along the veins where the virus is first moving out of the places of inoculation, the virus concentration is particularly high and produces an initial shock, causing some necrosis. Further studies on this problem would be of interest.

Robbins (*46*), who considered malformations and necrosis of tips of leaves in mosaic sugar beets to be symptoms of mosaic, sectioned such leaves and found in them necrosis of phloem and other tissues. From these observations he concluded that beet mosaic induced phloem necrosis. The malformations and tip necrosis that he described are not, however, symptoms of beet mosaic and frequently occur in plants not affected by this disease.[5]

While the condition of the phloem in mosaic beets was being considered, this tissue and others were examined also for the bodies described by Schaffnit and Weber (*48*) and Sirotina (*54*) in mosaic and healthy beets. These "elytrosoma" are said to be commonly spindle-shaped or very much elongated, often of the same size as the nuclei or larger. They either are homogeneous or they contain spheroidal granules. Schaffnit and Weber emphasized that these bodies occurred in undifferentiated phloem of young leaves. In the present study no bodies of the sort were observed. Plate 2, *A*, illustrates a tangential longitudinal section of procambium and young phloem of a bundle from a young sugar-beet leaf that showed severe mottling. There are no foreign bodies in evidence. The somewhat elongated nuclei are the only conspicuous structures within the procambial cells in the center of the field. In some cells two nuclei appear to be present, but only as the result of an optical effect. The procambial cells are radially compressed, and in tangential sections the nuclei of two cells may occur at very close levels. The procambial walls are not thick enough to obstruct the light materially. The phloem appears above and below the procambium in plate 2, *A*. This tissue has certain characteristic protoplasmic elements sometimes erroneously related to diseases (*19*, *21*). Plate 2, *B*, shows slimy accumulations of an immature sieve-tube element; plate 2, *C* and *D*, the sieve-tube plastids of mature sieve-tube elements. The "elytrosoma," as described in the literature (*48*, *54*), do not resemble the slime bodies or the sieve-tube plastids. They may be compared with some nuclei in the elongated cells of the vascular bundles, such as those in plate 2, *E* to *G*, but are not supposed to show the same internal structure as the nuclei.

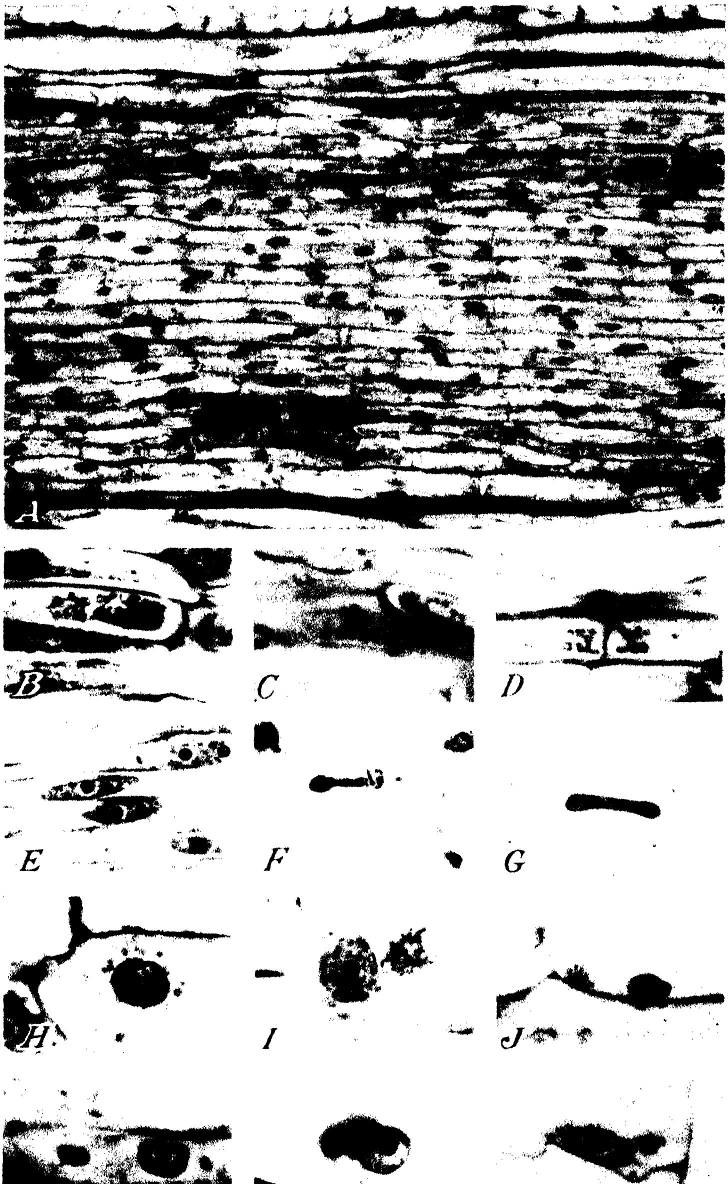
---

[5] Personal communication by Dr. C. W. Bennett and Dr. Eubanks Carsner, Division of Sugar Plant Investigations, United States Department of Agriculture.

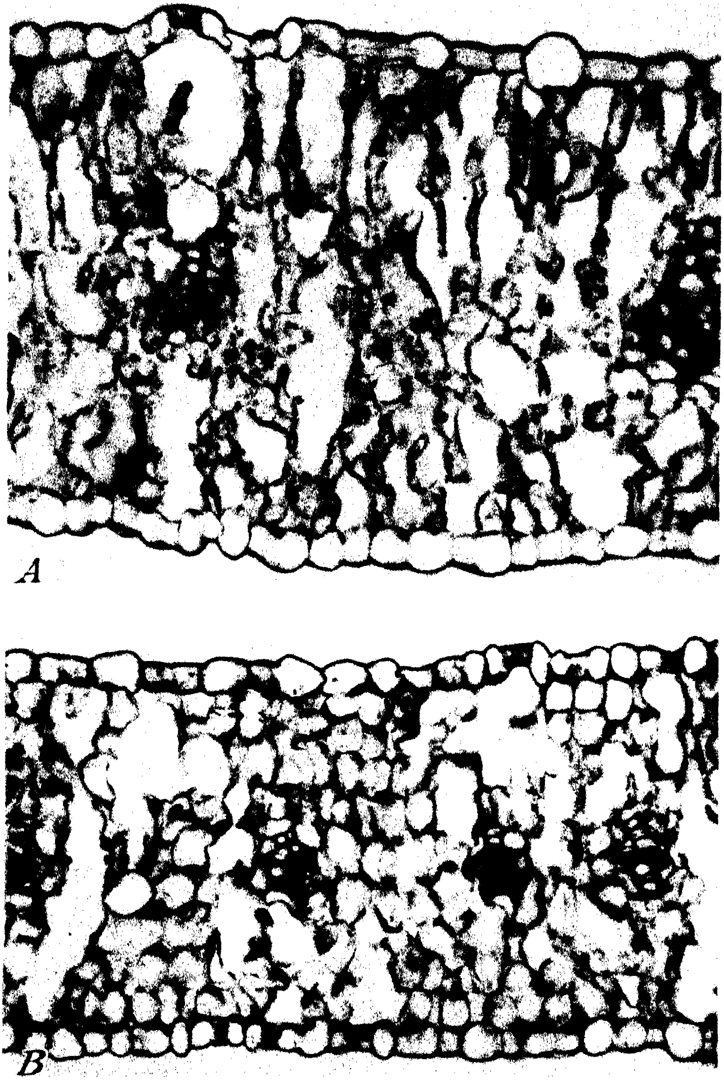
EFFECT OF MOSAIC UPON THE MESOPHYLL

According to Böning (*7*), the beet mosaic resembles other mosaics in that it induces mottling by retarding the differentiation of isolated spots in the mesophyll. These spots become the yellow areas as the leaf develops. The yellow areas are thinner, with fewer intercellular spaces and less chlorophyll, than the green areas. Böning (*7*), who attributed all these abnormalities to underdevelopment of the mesophyll and chloroplasts, recorded no destructive changes in mosaic leaves.

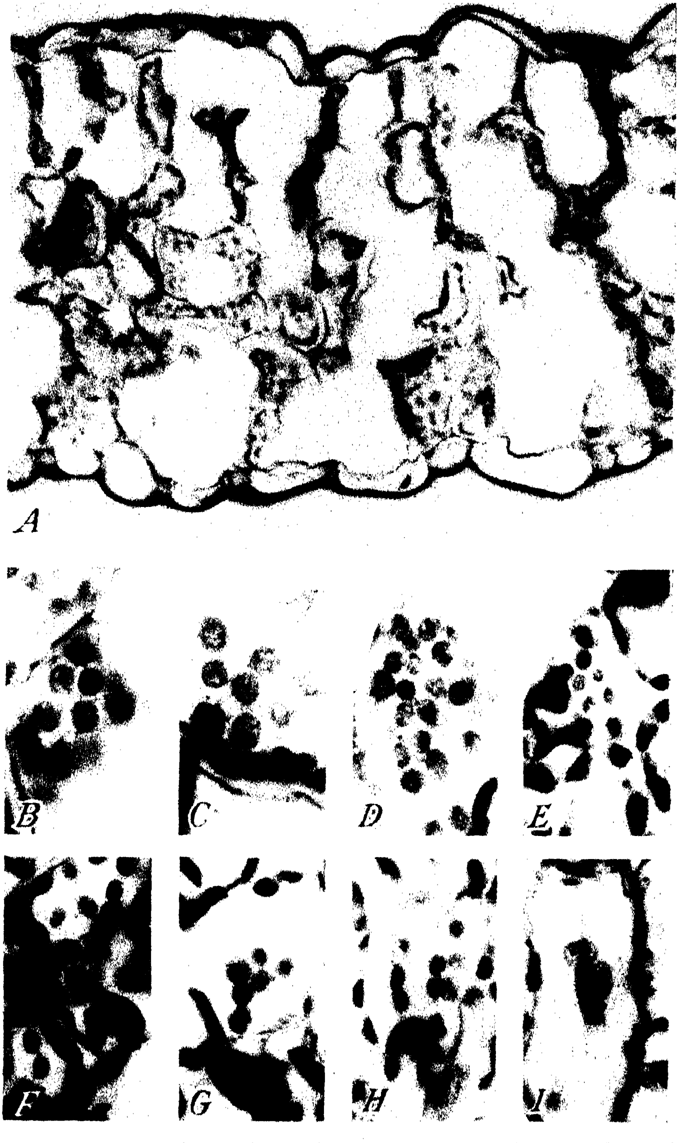
The material used in the present study confirms the view that the mesophyll in the yellow areas is hypoplastic. Plate 3 illustrates a section from a green area (*A*) and a yellow area (*B*) of the same medium-sized mosaic leaf. In each photograph the palisade region appears above, the spongy below. The yellow area, as contrasted with the green, shows certain juvenile characteristics: the mesophyll is not differentiated into palisade and spongy layers, its cells are more or less isodiametric, and the intercellular system is rather poorly developed. Its chloroplasts, furthermore, are indistinct—a feature considered in detail elsewhere in this paper. A dark-green area (pl. 3, *A*) may appear thicker than healthy mesophyll from leaves of comparable age or older (pl. 4, *A*); that is, the green areas may be hyperplastic. The degree of difference between them and healthy mesophyll varies considerably, however; they may be no thicker than the healthy leaf. The differences between the yellow and the green areas also vary. In a very young leaf the difference in the rate of development of the green and yellow areas will not have had time to produce variations in leaf thickness. In old leaves, on the other hand, the two areas may have the same thickness because the effect of the disease is insufficiently severe. According to Böning (*7*), the yellow areas continue to develop so that eventually they become more nearly



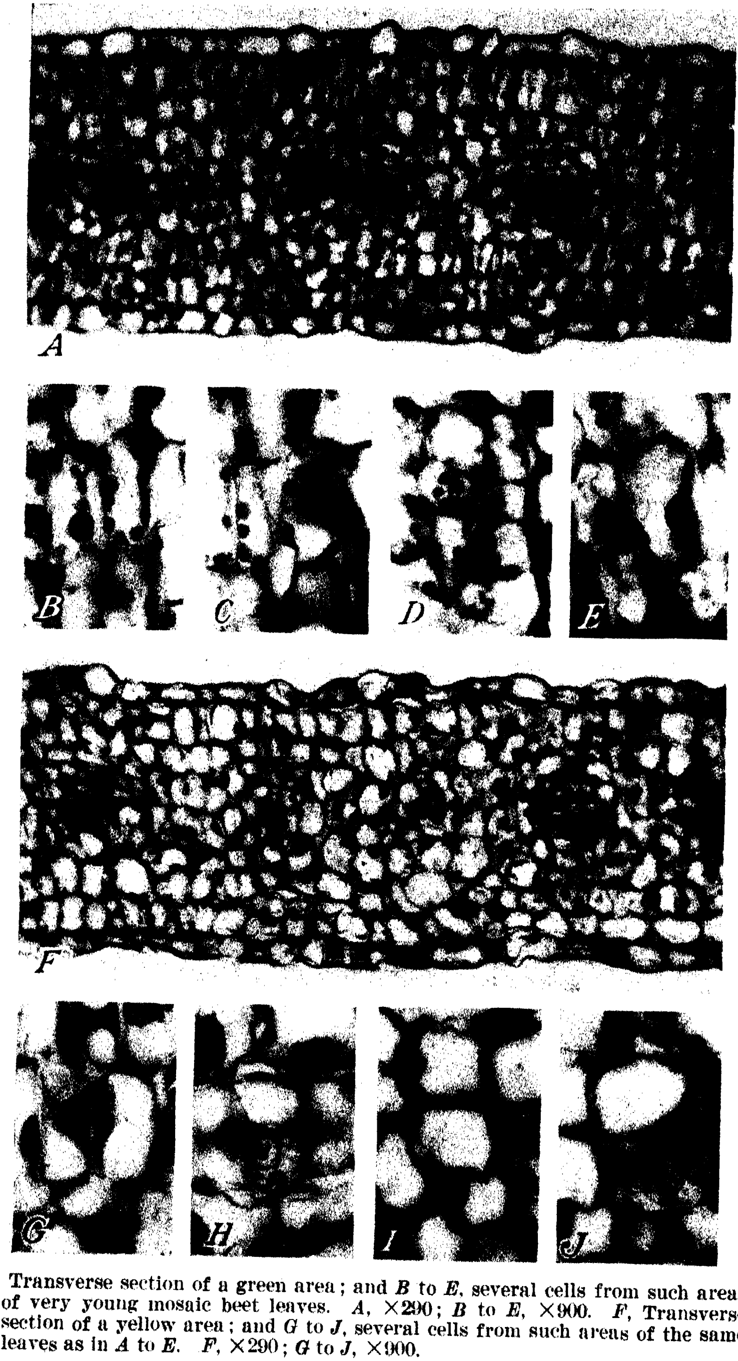
A, Tangential longitudinal section through procambium of a vascular bundle of a young mosaic-diseased beet leaf. × 290. *B*, Sieve-tube slime; *C* and *D*, sieve-tube plastids; and *E*, procambial nuclei from section as in *A*. *F* and *G*, Nuclei in xylem parenchyma of mosaic beet leaves. *H* to *M*. Plastids in different stages of coalescence and nuclei from various cells in mosaic beet leaves. *B* to *M*, × 900.



Transverse sections of a green (*A*) and yellow (*B*) area of a medium-sized beet leaf severely affected by mosaic. ×290.



A, Transverse section of mesophyll of healthy medium-sized beet leaf. ×290. *B* to *H*, Mesophyll chloroplasts, and *I*, epidermal plastids and nucleus from healthy (*B* to *F*) and symptom-free (*G* to *I*) beet leaves. *B* to *F*, fixed according to Navashin; *G* to *I*, according to Champy. *B* to *I*, ×900.



A, Transverse section of a green area; and *B* to *E*, several cells from such areas of very young mosaic beet leaves. *A*, ×290; *B* to *E*, ×900. *F*, Transverse section of a yellow area; and *G* to *J*, several cells from such areas of the same leaves as in *A* to *E*. *F*, ×290; *G* to *J*, ×900.

like the green. Some old leaves with mild mottling used in the present study showed only slight histologic differences between the yellow and green areas, but these leaves might have been infected while they were partly differentiated and in consequence might have been little affected by the virus. Whether a leaf showing as striking a difference between the light and dark areas as in plate 3 would largely eliminate this difference during further development still remains to be determined. Often the yellow areas show such profound cytologic disorganization that the possibility of recovery seems doubtful. Developmental studies with repeated samplings of the same leaves are desirable in this connection.

In leaves developing after the systemic infection has occurred the future yellow areas are characterized by suppression of cell divisions, whereas the cells of the future green areas continue to divide actively or perhaps even divide at abnormally high rates if the resulting green area becomes hyperplastic at maturity. In the early stages of development, consequently, the green area (pl. 5, *A*) has smaller but more numerous cells and appears less differentiated than the yellow area of the same leaf (pl. 5, *F*). Later, when the green area assumes its more or less mature characteristics, the yellow area appears as an underdeveloped portion of the leaf (pl. 3).

EFFECT OF MOSAIC UPON THE PLASTIDS

PLASTIDS OF HEALTHY AND SYMPTOM-FREE LEAVES

Plastids easily react to various stimuli by changes in their appearance, and the modifications within the range of normal variability are not clearly separated from those indicating a pathologic state. Because the problem of changes in the physical state of plastids is so complex (*29*, *52*, *61*), the plastids of the healthy and symptom-free beets were studied in detail before diseased material was considered.

When plastids were examined in fresh sections, two criteria were used in identifying living cells: the occurrence of protoplasmic streaming and the presence of mitochondria. Though the absence of streaming does not necessarily indicate that the cell is dead, its presence is generally regarded as a sign of life. Mitochondria, on the other hand, are very fragile; their presence indicates that the cell has not been materially injured in preparation. These bodies were not identified by applying specific tests; rather, they were located and interpreted as mitochondria by comparing published descriptions of mitochondria with bodies observed in cells of a variety of plants, including the beet. Furthermore, different fixatives were applied under the microscope. Some were acetic acid fixatives, known to cause a dissolution of mitochondria; others were bichromate fixatives, said to preserve them. The mitochondria as identified by these means in the beet commonly appear as dumbbell-shaped rods with swellings on both ends. Frequently the elongated mitochondria separate into pairs of spherical bodies. With time this separation becomes more common, so that after 2–3 hours of observation the spherical bodies predominate. The mitochondria are carried by the streaming cytoplasm and often show also an oscillatory movement. Other common bodies comparable in size with mitochondria occur in the living cells. These, being much more highly refractive than the mitochondria, are probably oil globules.

The appearance of the nucleus may also be used as a criterion of cell condition. In a living cell, with ordinary illumination, the nucleus shows no structure; but in a dead cell it has a granular reticulum or is granular throughout.

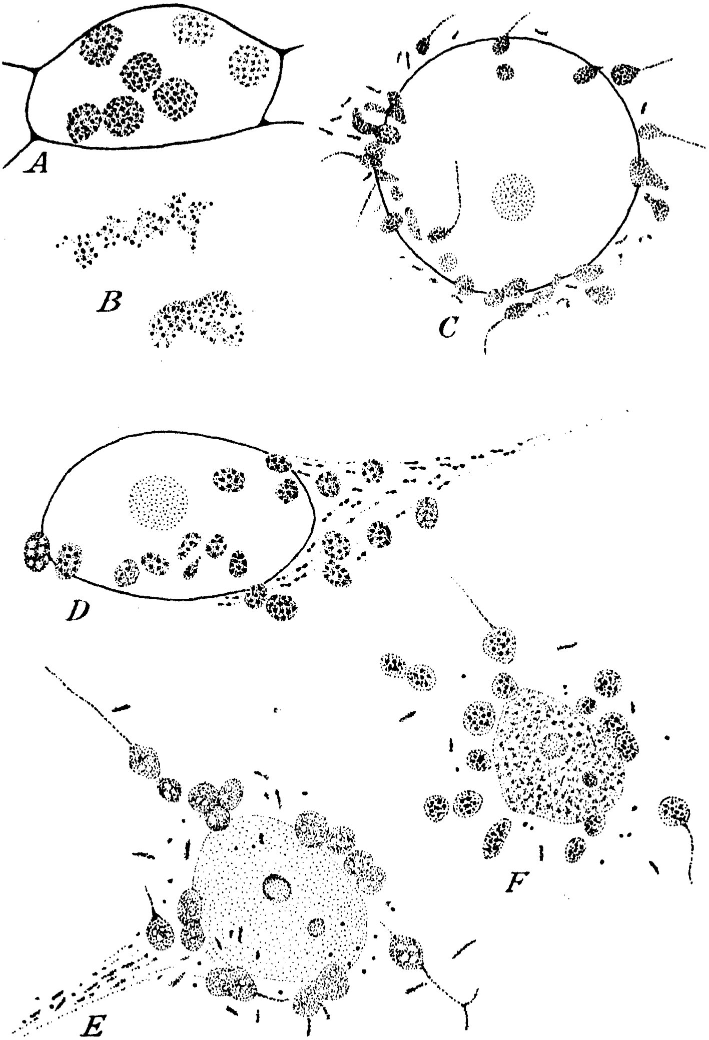


FIGURE 3.—Plastids and nuclei in freehand sections from mosaic-free beet plants. *A*, Chloroplasts of a mesophyll cell. × 1,360. *B*, Chloroplasts crushed by pressure on cover glass. × 1,360. *C*, Leucoplasts and nucleus from pith of hypocotyl. × 1,400. *D*, Chloroplasts and nucleus from petiole. × 1,400. *E*, Chloroplasts and nucleus from pith of seedstalk. × 1,400. *F*, Same as in *E* after treatment with a bichromate fixative. × 1,400

According to recent literature, chloroplasts may appear either granular or homogeneous, both conditions being normal (*4, 31, 60, 62, 63*). The chloroplasts of the beet leaves, whether from the mesophyll (fig. 3, *A*) or from other chlorophyll-containing parenchyma (fig. 3, *D*), often show a distinctly granular structure. The granules are arranged in somewhat orderly fashion (fig. 3, *A*); they appear dark at one focus, light at another (fig. 3, *D*); and when the plastid is mechanically crushed by pressure on the cover glass, the mass remains granular (fig. 3, *B*). At other times the chloroplasts are very finely granular, with few, somewhat large granules showing no orderly arrangement (fig. 5, *A–C*). No entirely homogeneous chloroplasts were observed in this study.

The chloroplasts of the mesophyll may be more or less flattened and appressed to the walls. Sometimes they are spread out so much as to crowd each other and assume somewhat angular outlines (fig. 5, *B*). Or they may be rounded off and not at all crowded (fig. 3, *A*; 5, *C*). Different cells in the same section may show different degrees of flattening of the chloroplasts. Though these differences in shape might partly account for the apparent variations in plastid size, actual differences in plastid mass probably occur also. The photomicrographs in plate 4, *B* to *H*, illustrate variations in size of chloroplasts upon fixation and dehydration in sections of mature leaves. The plastids in plate 4, *B* to *D*, were found in different cells of one leaf section; those in plate 4, *E* and *F*, in cells of another; those in plate 4, *G* and *H*, in cells of a third. Even within a given cell, plastids may vary in size (fig. 5, *A*). Apparently in relation to the time of collection, the chloroplasts either did or did not contain starch grains. The chloroplasts in plate 4, *B* to *F*, show starch grains.

The chloroplasts of the parenchyma cells surrounding the vascular bundles of the medium and large veins are smaller and paler than the chloroplasts of the mesophyll. They tend to aggregate about the nucleus (fig. 3, *D* and *E*) like the leucoplasts that occur in the colorless pith parenchyma of the root crown or flowering stalk (figs. 3, *C*, and 4, *A*). The aggregation of certain plastids around the nucleus has been recorded in the literature (*29, 52*). In living cells the leucoplasts or the pale chloroplasts appear to touch the nucleus (figs. 3, *C* to *E*). When killed, however, the nucleus shrinks, and the distance between it and the plastids is somewhat increased. Figure 3, *F*, shows the same nucleus and plastids as figue 3, *E*, but after treatment with Navashin's fixing fluid. In the fixed cell of figure 4, *A*, also, the nucleus is somewhat shrunken.

The leucoplasts characteristically undergo amoeboid changes in shape. Figure 4, *B*, shows the changing shape of one leucoplast during 15 minutes. The amoebid shape of leucoplasts is also evident in figure 3, *C*, taken from a living cell, and in figure 4, *A*, showing a cell treated with a mitochondrial bichromate fixative. Though the chloroplasts display less tendency toward amoeboid changes, they may have colorless processes that alter their shape and size (fig. 3, *E*).

The living leaf sections are eventually injured by the tap water used for mounting them. Sooner or later after being mounted, certain cells exhibit vesiculation of plastids. The plastids (and the mitochondria) swell and are converted into vesicles while the nucleus shrinks (fig. 4, *C*). When a chloroplast swells, it sometimes appears as though split in two halves still attached to the vesicle (fig. 4, *D*); or a green granular mass dispersed over the surface of the vesicle makes the latter appear in optical section like a ring with green particles adhering to it all around. Whether these particles are within the membrane of the vesicle and form bulges on its surface or whether they adhere on the outside has not been ascertained. The literature does not clearly answer this question (*36*, *41*, *61*). One can fix the vesicles by treating the section with a fixing fluid under the microscope

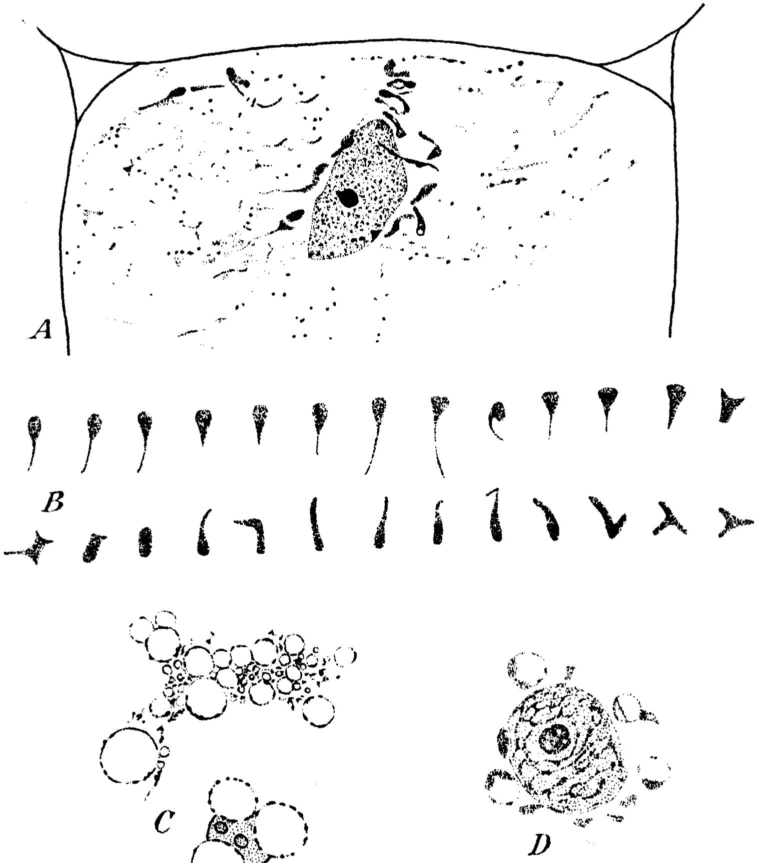
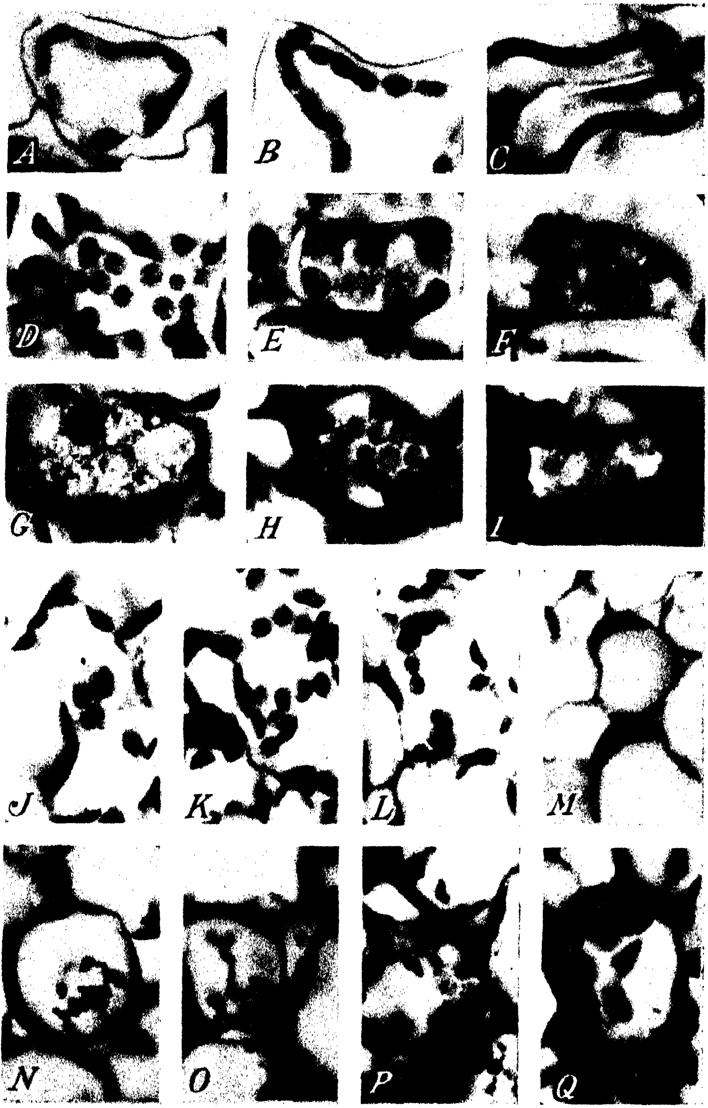


FIGURE 4.—Nuclei and plastids from mosaic-free beet (*A–C*) and *Chenopodium murale* (*D*) plants. *A*, Protoplast of parenchyma from hypocotyl treated with a bichromate fixative and embedded in paraffin. × 1,200. *B*, Series of freehand drawings of the same living leucoplast from parenchyma cell, showing amoeboid changes that occurred during 15 minutes. About × 1,200. *C*, Shrunken nucleus and swollen chloroplasts and mitochondria affected by tap water used for mounting. × 1,200. *D*, Shrunken nucleus and swollen chloroplasts treated with a bichromate fixing solution after they were affected by tap water. ×1,200



A and *B*, Chloroplasts from a healthy leaf. *C*, *E* to *I*, and *M* to *Q*, Cells from yellow areas of mosaic beet leaves. *D*, and *J* to *L*, Cells from green areas of mosaic beet leaves. *A* to *I*, Fixed according to Navashin; *J* to *Q*, according to Champy. All × 900.

(fig. 4, *D*) ; but they have not been observed in paraffin material. In fresh sections they eventually burst, leaving a greenish granular mass behind. When during cutting the chloroplasts are extruded from the cells, they vesiculate much quicker than the plastids within the cells. There is also a variation in the time when this phenomenon appears in different cells of the same section. In some cells, probably injured in cutting, vesiculation appears at once; in others not for various lengths of time; or the protoplast may disintegrate without swelling of plastids.

PLASTIDS OF MOSAIC LEAVES

The chloroplasts of the dark-green areas of mosaic leaves (fig. 5, *E* and *F*, and pl. 6, *D*, *J* to *L*) generally resemble the chloroplasts of healthy and symptom-free leaves (fig. 5, *A* to *C*; pl. 4, *B* to *H*). The two kinds of plastids show similar variations in size, degree of granulation, degree of spreading and flattening; both may or may not have starch. Plastids in sections of the green areas of mosaic leaves tend to show somewhat more pronounced vesiculation than the healthy mesophyll.

The chloroplasts of the yellow areas undergo pathologic changes of different degrees. Generally, if these chloroplasts are present as discrete bodies, they are light-colored. Starch is occasionally present if the plastids are not much modified. The latter apparently vary in size as much as the chloroplasts of the dark-green areas or of healthy leaves; but no statistical studies of plastid size were made during the present investigation. Chloroplasts in yellow areas show a much greater tendency toward vesiculation than those in healthy mesophyll or in dark-green areas of mosaic leaves. Numerous cells in water mounts of sections of yellow areas appear as though filled with foam (fig. 5, *G*), the vesicles sometimes deforming each other. More or less fragmented chloroplasts are associated with the vesicles. Eventually the latter burst, and a granular pale-green or yellowish mass remains.

Chloroplasts that do not become vesiculated tend to be spread out very thin or to coalesce. Sometimes they appear amoeboid in shape (fig. 5, *D*). The different appearance of the coalescing plastids suggests the following sequence in the process. The plastids are clumped within a cytoplasmic sheath that appears very tenuous (fig. 6, *A*). Then the chloroplasts lose their sharp outlines (fig. 6, *C*), and finally the plastids and the cytoplasm together appear as a greenish mass closely appressed to the cell wall (fig. 6, *B*). At this stage the cells still respond with plasmolysis to hypertonic sugar solutions. Eventually, in fresh sections, the coalesced material fragments. At the same time the nucleus becomes granular, and crystals appear in the cell. Some highly refractive bodies, usually one per cell, may be present before the cell becomes completely disorganized (fig. 6, *A*, above); but there may be similar bodies in symptom-free sections also. Usually the plastids appear in different stages of coalescence when the section is mounted, but sometimes fusion occurs during observation. Thus of the two cells in figure 6, *D*, the lower showed fusion of plastids about 30 minutes after the section was prepared. The plastids clumped together, became coarsely granular, and then fused (fig. 6, *E*). Cytoplastic streaming ceased, and crystals appeared near the nucleus. The slide was placed in a refrigerator overnight, and

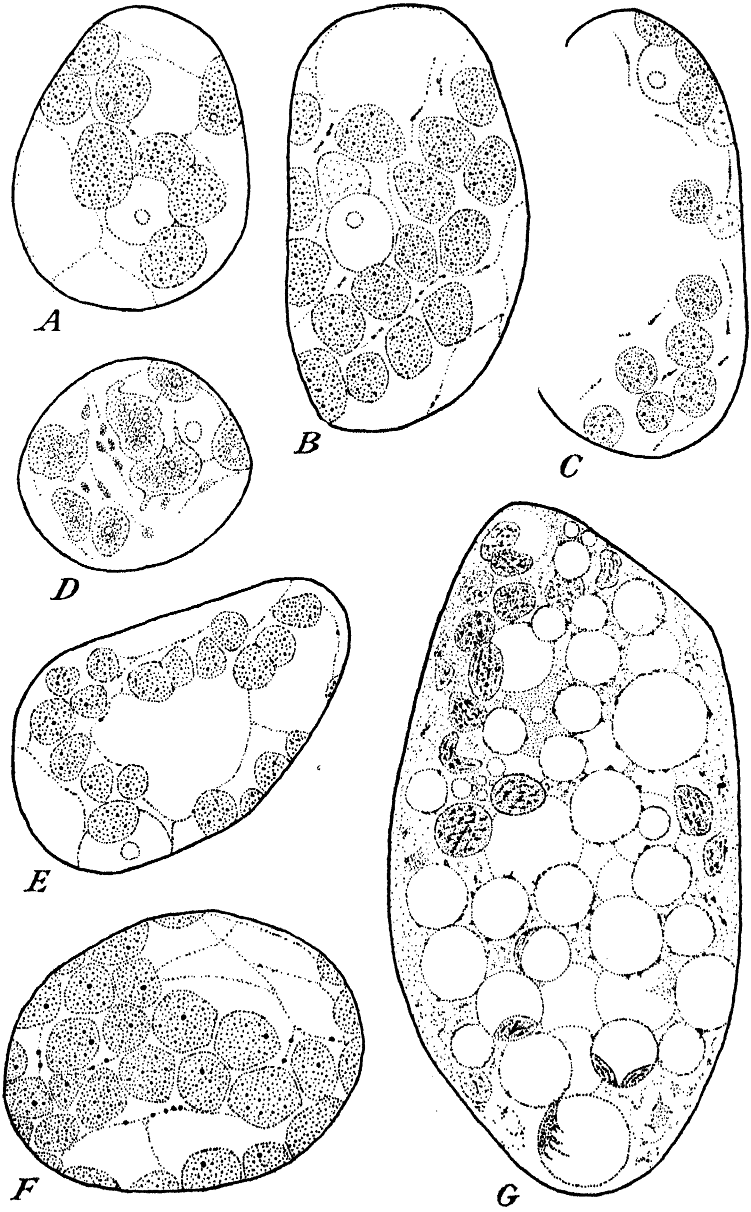


FIGURE 5.—Mesophyll cells from freehand sections of sugar-beet leaves mounted in tap water. *A* to *C*, From a plant free of symptoms; *D* and *G*, from yellow areas, and *E* and *F*, from green areas of mosaic leaves. Further explanations in text. All × 1,450.

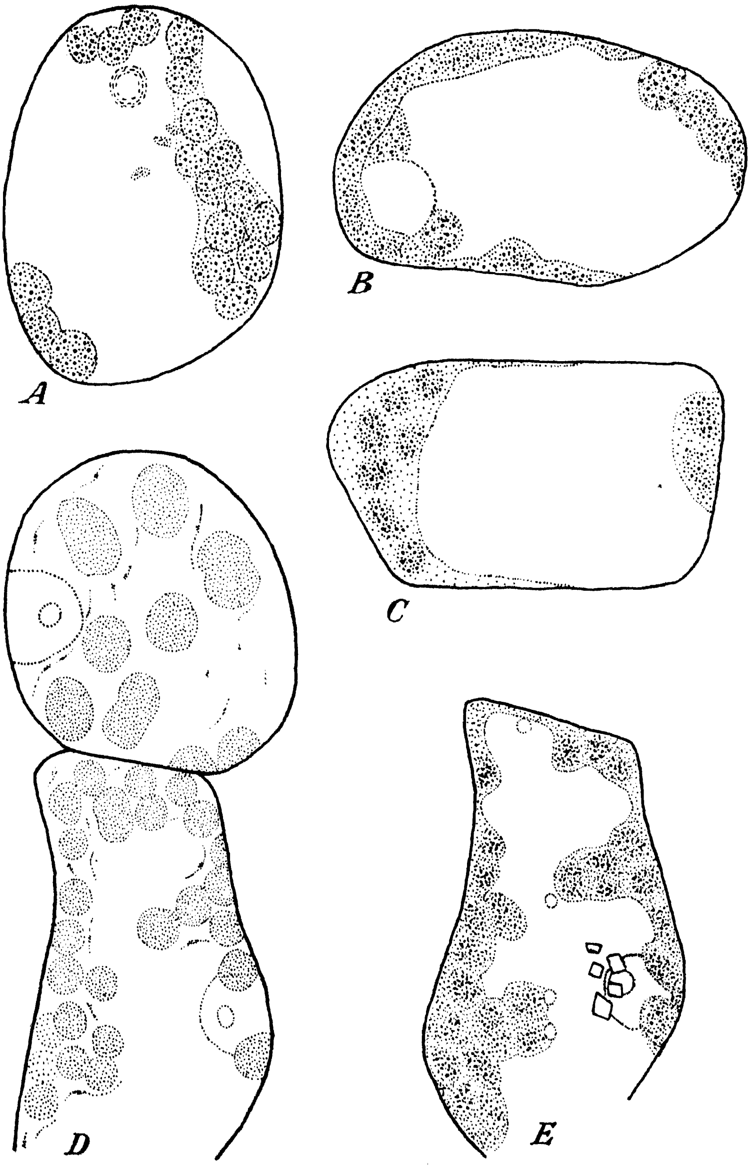


FIGURE 6.—Mesophyll cells from mosaic beet plants mounted in tap water. The chloroplasts in *A* to *C* and *E* are in different stages of coalescence. The chloroplasts in *D* are not coalesced, but the lower cell in *D* appeared as in *E* 30 minutes after being mounted. All × 1,450.

next morning the upper cell was still intact. In the lower cell, however, the protoplast was completely disintegrated. The two cells in figure 6, *D*, were originally drawn to illustrate the characteristic variation in chloroplast size in the mesophyll of mosaic and diseased beet leaves. The cells in figure 6, *D*, occurred in the border between a yellow and a green area.

Coalescence of chloroplasts in yellow areas is evident also in paraffin material. Plate 6, *C*, shows coalesced plastids within the cytoplasmic sheath lining the walls of two cells. Plate 6. *E* to *I*, gives several face views of partly and entirely fused chloroplasts. The spreading of plastid material in earlier stages of coalescence is obvious in plate 6, *E*, a view which strikingly contrasts with that of the rounded-off plastids from a dark-green area in plate 6, *D*. The occurrence of coalesced plastids in paraffin material and in parts of fresh sections somewhat removed from cells obviously injured in preparation indicates that the chloroplasts of yellow areas may be in various stages of coalescence in intact leaves.

Rather thoroughly degenerated cells such as are encountered in paraffin sctions of yellow areas appear in plate 6, *M* to *Q*. For contrast, cells from the dark-green areas of the same leaf are shown in plate 6, *J* to *L*. In plate 6, *M*, the chloroplasts, nucleus, and cytoplasm form together a protoplasmic sheath closely appressed to the wall. This sheath is rather thick where the nucleus occurs. The cell in plate 6, *N*, contains darkly staining deposits, probably tannic in nature, as well as the photoplasmic sheath lining the wall. The cell in plate 6, *O*, is similar except that it shows also a vesiclelike structure at the lower right. Another vesicle appears in the cell above. The origin of these vesicles has not been determined. They are probably not degenerated nuclei, because often more than one vesicle occurs in a cell, whereas the chloroplasts are part of the coalesced mass lining the wall. In plate 6, *P* and *Q*, the plastids appear like irregular amorphous masses smeared on the wall. The nuclei were not discernible in cells shown in plate 6, *N* to *Q*.

It seems doubtful that cells so disorganized as those in plate 6, *M* to *Q*, could recover and become more nearly like those of the dark-green areas (pl. 6, *J* to *L*). The literature indicates, furthermore (see Discussion), that fusion of plastids, sometimes called agglutination (*39*), is an irreversible process. The degenerative changes observed in the chloroplasts of the yellow areas, especially their pronounced tendency to swell and burst in water mounts or to fuse into amorphous masses, indicates a profound alteration in their physical state. The entire protoplast appears to be changed, since the cells in the yellow area usually show no shrinkage of protoplasts (pl 6, *C*, *M* to *P*) such as commonly appears in paraffin sections of healthy (pl. 6, *A* and *B*) and dark-green cells (pl. 6, *L*).

Since the partly or completely coalesced chloroplasts of diseased cells stain lightly, in photography the contrast must be artificially increased in order to bring out the plastids, as in plate 6, *P* and *Q*. The diffuse condition of the plastids and their light staining explains the difference in cytological appearance between the dark-green and the yellow areas. In the former the chloroplasts are discrete, more or less deeply stained bodies (pl. 3, *A*) ; in the latter they are obscure (pl. 3, *B*).

The degree of chloroplast degeneration in yellow areas varies in different leaves and also in different parts of the same leaf or even of the same yellow area. These differences might be caused by a delay in virus entry into some areas or cells as compared with others, or by variations in resistance of cells to the virus. A combination of the two factors might be assumed also. There is evidence, however, that the mosaic virus affects the chloroplasts in different stages of development. Scarcity of plastids and suppression of cell division clearly differentiate the yellow (pl. 5, *F–J*) from the green (pl. 5, *A–E*) areas in leaves that show the earliest signs of mottling. Judging from the youngest sections in plate 5, *B* and *G*, the chloroplast development might be suppressed or delayed in the yellow areas. At least, it is difficult to decide whether the appearance of cells as in plate 5, *G*, results from inhibition of chloroplast development or from disorganization of structures already present in the cells. According to Sheffield (*53*), plastid primordia usually are destroyed in solanaceous hosts having aucuba mosaic, whereas mature plastids are not affected by the virus. No special attempts to distinguish plastid primordia from plastids proper were made in the present study. Certainly, however, the yellow areas are deficient in plastids at the earliest stages of their differentiation (pl. 5, *G*). Slightly later (pl. 5, *H* to *J*) some plastids develop, but they stain lightly and are somewhat diffuse. In still older leaves the yellow areas may have more or less affected chloroplasts (pl. 6, *E*, *F*, *H*), or the latter may be thoroughly disorganized (pl. 6, *G*, *I*, *M*, to *Q*). Probably the more severely degenerated cells are those affected by the virus in their earliest stages of development.

The evidence that choloroplasts of partly developed leaves are affected by the mosaic virus was obtained from leaves that were inoculated or that received the virus from the inoculated leaf in advanced stages of development.[6] Yellow spots sometimes develop at the place of inoculation. In the present study such spots showed signs of injury, apparently caused by rubbing the leaves during inoculation; the epidermal cells on the upper side of the blade and sometimes also the deeper layers of mesophyll were necrotic, and wound-healing reactions had set in beneath the necrotic cells. As is well known, wound-healing involves hypertrophy and hyperplasia accompanied by loss of chloroplasts, if the latter are present in the affected tissue. Many other cells, however, in the yellow areas somewhat removed from the places of superficial necrosis also showed degeneration of chloroplasts. The latter appeared very small or diffuse or were fragmented, or the entire protoplast was replaced by flocculent material. Darkly staining bodies of cholorplast size, possibly necrotic chloroplasts, also occurred in these sections; and some entirely necrosed cells were dispersed among those that were still intact. Similar degenerative changes occurred in the yellow strips of mesophyll that were located along the larger veins through which the virus moved out from the place of inoculation; also in the yellow mesophyll associated with a lateral vein in a leaf that was in an advanced stage of development when the virus entered the plant through another leaf. All the sections mentioned above were compared with similar sections of mesophyll from symptom-free parts of the same leaves. These showed no plastid abnormalities. Plate 7, *A*, illustrates a section of yellow mesophyll occurring along a lateral vein through which the virus passed when the leaf was partly grown. The scarcity of chloroplasts strikingly contrasts this section with the mesophyll in plate 7, *B*, taken from a similar position in the opposite, symptom-free side of the same leaf. The observations just given indicate that cells in different stages of differentiation may be affected by the virus.

---

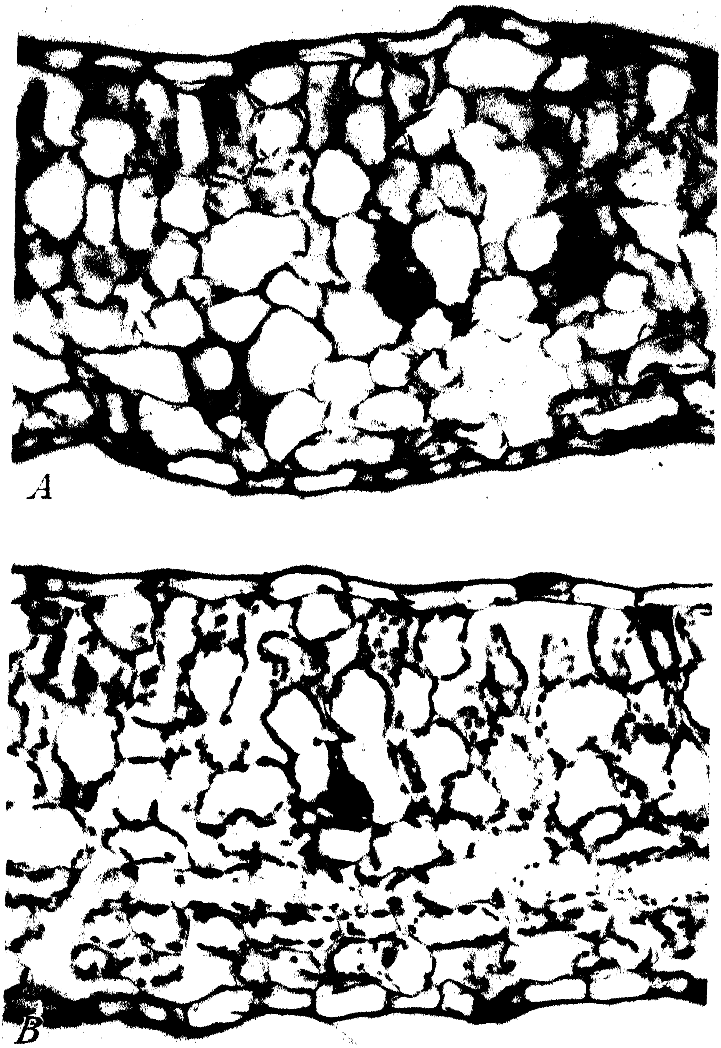
[6] This material was prepared by Dr. C. W. Bennett.

Though the chloroplasts of the mesophyll show the most striking effect of the mosaic, the plastids of the parenchyma along the larger vascular bundles apparently also become disorganized. Such cells sometimes contain flocculent or reticulate bodies that lie more or less close to the nuclei (pl. 2, *I*, *J*, and *M*). These bodies may also occur in parenchyma cells of the vascular bundles (pl. 2, *K*) and in epidermal cells (pl. 2, *L*). The bodies seemingly result from coalescence of plastids. As already mentioned, plastids of some kinds of parenchyma and of the epidermis frequently aggregate near the nuclei (pls. 2, *H*, and 4, *I*). This tendency would explain the proximity of the bodies to the nuclei. Views like the one in plate 2, *I*, suggest transitional stages between the aggregation of plastids near the nucleus and their fusion into a body. The absence of plastids in cells containing the bodies is still another indication that these bodies are related to plastids. Morphologically the bodies in mosaic beet parenchyma resemble the X-bodies described in other mosaic diseases.

DISCUSSION

In general, the results of the present study of the mosaic sugar beet agree with other workers' conclusions that the yellow areas in the mottled leaves of plants affected by mosaic viruses are deficient in chlorophyll and tend to be more or less thin as compared with the dark-green areas (*7*, *8*, *9*, *14*, *15*, *25*, *32*, *42*, *55*, *65*, *66*). The mosaic beet, furthermore, in common with many other mosaic plants (*9*, *14*, *42*, *65*, *66*), shows certain juvenile characteristics in the yellow mesophyll: the latter is not differentiated into palisade and spongy parenchyma and its cells are cuboidal in shape and rather closely packed. In other words, these areas are underdeveloped, or hypoplastic, as compared with the green areas of the diseased leaves and with the mesophyll of the healthy leaves. Usually the yellow areas are described also as thinner than healthy mesophyll. An exception appears to be mosaic *Datura stramonium*, in which, according to Smith (*55*), the yellow areas are approximately as thick as the healthy mesophyll. In the mosaic beet the yellow areas, though tending to be comparatively thin, varied in thickness and were sometimes as thick as the green areas of the same leaf or as the mesophyll of healthy leaves.

The dark-green areas of mosaic leaves are often described as thicker than healthy leaves (*14*, *15*, *42*, *55*). Unusually long, narrow palisade cells are mentioned as occurring in these areas. Goldstein (*25*), on the other hand, regards the dark-green areas as hypoplastic, though less so than the yellow areas. According to Clinch (*9*), the green spots of potato leaves affected with crinkle are thicker than healthy leaves, but in potatoes affected with certain other mosaics they are of the same thickness as the healthy mesophyll. In the present study the green areas of some mosaic beet leaves were markedly



A, Yellow mesophyll occurring along a vein through which the mosaic virus passed when the leaf was partly grown. The chloroplasts have been partly destroyed in this mesophyll. × 290. *B*. Mesophyll taken from a similar area on the other side of the same leaf as in *A*, the side that showed no mosaic symptoms. A normal complement of chloroplasts appears in this section. × 290.

thicker than healthy mesophyll of similar age, whereas in other leaves the green mesophyll resembled the healthy.

According to the review just given with regard to thickness of mesophyll, the degree of difference between the yellow and the green areas on the one hand and between the healthy and diseased leaves on the other may vary considerably. Workers have also presented conflicting data on the severity of cytologic effects of mosaic viruses, such as abnormal starch accumulations (*7, 9, 16, 34, 42, 53, 57, 65, 66*), or the inhibition of plastid development, or the occurrence of chloroplast destruction (*7–15, 25, 32, 34, 42, 44, 53, 57*). Such variations may be expected. Viruses naturally differ in virulence, and the severity of symptoms is affected by the age of the leaf at the time of infection. Thus Stone (*57*) found little effect of the mild mosaic virus upon the leaf thickness in potato, whereas Hoggan (*32*), using other mosaic viruses, observed the usual reduction in thickness of the yellow areas in solanaceous hosts. As Goldstein's (*25*) study, on the other hand, clearly shows, leaves infected when still very young exhibit more marked external symptoms and internal abnormalities than those that were partly differentiated when the virus entered them. Various environmental conditions also affect the expression of symptoms, as is evidenced by the masking of symptoms that occurs under certain conditions of light and temperature in many mosaics. The divergent reports on starch accumulation must be judged in the light of the observations that viruses delay both accumulation and removal of chloroplast starch. The susceptibility of symptom expression to various internal and external factors might well explain the conflicting data on the anatomic and cytologic abnormalities in mosaic diseases.

As was observed in the introduction to this paper, some workers attribute the abnormalities of the yellow areas of mosaic leaves to underdevelopment only; others describe destructive changes in the affected protoplasts as well. The present study supports the view that the cell contents are disorganized in mosaic leaves. Clinch's (*9*) conclusion that definite physiological disturbances act in conjunction with an initial underdevelopment seems to picture correctly the abnormalities in the light areas of mottled leaves.

Hypoplasia in yellow areas merits further attention from students of pathological anatomy. In partly or fully developed leaves the yellow mesophyll usually appears more juvenile than the green. Judging from certain developmental studies on mosaic plants (*12, 14, 34*), cell division in early stages of differentiation of mosaic leaves is suppressed in the yellow areas. Similar observations were made in the present study. Iwanowski (*34*), furthermore, pointed out that the yellow areas precociously develop intercellular spaces, so that in young leaves these parts seem to consist of a tissue older than the still-dividing, compact green mesophyll. Grainger and Heafford (*27*) found that certain groups of mesophyll cells in leaf primordia of mosaic tobacco underwent earlier vacuolation than other adjacent ones; they suggested that these cells would become the yellow areas in the mature leaf. This suggestion, if correct, would indicate that the cells in the yellow areas complete differentiation before those of the green. The early appearance of intercellular spaces and the suppression of cell divisions in these areas point in the same direction. Apparently, then, in the early stages of differentiation, the yellow areas are hyperplastic, since certain phenomena of tissue maturation appear in them earlier than elsewhere in the mesophyll. Later they appear hypoplastic because they do not complete the typical differentiation of the mesophyll tissue.

Certain workers interpret the mottling of mosaic leaves as the result of an uneven spread of virus in the affected leaves (*12*, *25*, *53*). Light areas arise in those parts of the leaf that are still very young when invaded by the virus, whereas green areas develop if the virus is late in reaching the cells. Despite good evidence that yellow areas contain a higher virus concentration than the green leaf parts (*26*, *33*), other phenomena besides the uneven spread of the virus might cause the mottling in mosaic leaves. Cells within the same tissue, even those occurring side by side, may react differently to the same stimuli or to injurious agents. Alexandrov (*1*) found that the tendency of sunflower chloroplasts to swell under certain climatic conditions varies within the same part of the leaf. According to Beauverie (*3*), fusion and degeneration of epidermal chromoplasts in *Ranunculus* petals treated with saponin may occur in one cell and not in another adjacent to it. Finding that certain zones die off in leaves of *Bryum capillare* treated with α-rays, while others remain alive, and that there are no transitions between the two zones, Biebl (*6*) assumed that the dead parts were less resistant. Gratzy-Wardengg (*28*) observed that starvation caused the plastids to disintegrate in most cells of fern prothallia, but that certain "green islands" of cells retained their chloroplasts. These cells showed particularly high osmotic values and were able to undergo regenerative changes. According to Schwarz (*51*), *Selaginella* grown at temperatures of 10° C. and below develops variegated leaves, the plastids degenerating in the white areas. Islands of green cells may remain, however, within the white tissue, indicating that individual cells vary in their responses to low temperatures. Sometimes one of two guard cells of the same stoma may remain green while the other turns white. At the present stage of our knowledge of the virus-host relations, we cannot ignore the possibility that cells of the same age and location in the plant body vary in susceptibility to the virus.

With regard to the plastid abnormalities observed in the mosaic beet leaves, both vesiculation and fusion of plastids have been described by many workers. These abnormalities may be induced by various factors. In general, living plastids respond easily with vesiculation to changes in the osmotic equilibrium (*2*, *4*, *29*, *38*, *49*, *61*). The swollen vesiculated chloroplasts may endure for many days, reacting to hypertonic and hypotonic solutions with changes in volume (*36*, *39*). As a rule, the vesiculated plastids eventually burst, leaving behind a greenish granular mass. Knudson (*37*), however, reported having obtained a stable form of vacuolated plastids in *Polypodium aureum* gametophytes that occurred in progenies from X-rayed spores.

Several workers have recorded vesiculation of chloroplasts in fresh sections of mosaic-diseased leaves. Iwanowski (*34*), who found that vesiculation in mosaic tobacco occurred most rapidly in the yellow areas, took this fact to indicate a lower resistance of these plastids. Similarly, Hoggan (*32*) saw plastids of the yellow leaf parts in mosaic potatoes swelling into colorless vesicles. She suggested that this phenomenon might bear on the formation of spheres in tomato mosaic reported by Sorokin (*56*). The liquefaction of the chloroplasts described by Eckerson (*17*) in tomato mosaic also somewhat resembles vesiculation. According to Beauverie (*2*, *4*), vesiculation of chloroplasts in potatoes affected with leaf roll becomes more pronounced with increase in severity of the symptoms. Cook (*12*) and Sheffield (*53*), observing vesiculation of chloroplasts in healthy and virus-diseased leaves, concluded that it had no bearing on the diseases. The results of the present study—observations on the rapid vesiculation and disintegration of chloroplasts in the diseased leaves and on the relatively greater stability of the chloroplasts in the symptom-free leaves—support the concept that resistance of the plastids to changes in the osmotic conditions of the environment is reduced by the presence of the virus.

The spreading of plastids, their loss of characteristic shape, the clumping and fusions observed in the mosaic-diseased beet leaves, all indicate a physiological disturbance. Similar plastid abnormalities have been frequently described in the literature. Although constituting pathologic phenomena, they are not sharply separated from normal variations in shape and consistency of plastids. A spreading and flattening of chloroplasts, so that they crowd each other and become angular in outline, is apparently a normal state of the chloroplasts in the mesophyll, which, however, alternates with a rounding off in relation to changes in light, temperature, moisture, and other conditions (*6*, *52*, *64*). Amoeboid changes in shape with the production of pseudopodialike processes are known to occur in chloroplasts and particularly in leucoplasts under apparently normal conditions (*23*, *29*, *31*, *38*, *39*, *49*, *52*, *61*). Plastids may produce pseudopodia in light and round off in darkness (*45*). Pseudopodia may be formed by plastids when the cells are exposed to X-rays or are treated with plasmolyzing agents (*6*, *24*, *37*, *39*).

Spreading and change in the shape of plastids, however, may be the beginning of pathologic changes that result in their coalescence and final breakdown (*3*, *6*, *28*, *39*, *41*). The fusion of plastids into an apparently viscous mass, sometimes called agglutination (*39*, *41*), has been induced by treatment with hypotonic and hypertonic solutions, alcohols of low percentages, other organic substances, α-rays, and neutral red (*3*, *6*, *39*, *41*, *59*, *61*). Though agglutination is usually described as an irreversible pathologic change, Knudson (*37*) reported agglomerated and fused plastids as stable forms in gametophyte progenies from spores of *Polypodium aureum* treated with X-rays.

Clumping and fusion of plastids have rarely been recorded by students of mosaic diseases. Dickson (*14*) spoke of agglomerations of chloroplasts and coalescence into irregular green masses in severe mosaic infections. Nelson (*44*), describing bean mosaic, mentions flattening of the stroma of chloroplasts and their eventual collapse into a coherent mass of viscous, pale-yellow or colorless material with a highly refractive surface. Loss of contours, clumping and fusion of plastids, were also observed in noninfectious chlorosis and variegations (*30*, *50*, *51*).

Judging from the data on plastid abnormalities, these structures can react similarly to many different injurious agents and environmental effects. The vesiculation and coalescence of chloroplasts in mosaic leaves cannot, therefore, be regarded as responses to a specific action of the mosaic virus. They are symptoms of physiologic disturbances within the diseased cells—disturbances that might be brought about by various agencies, as well as viruses, and which reduce the stability of the plastids.

SUMMARY

Sugar-beet leaves with systemic symptoms of mosaic used in the present study showed no abnormalities in the phloem; this tissue was not degenerated, nor did it contain any foreign or abnormal bodies. Apparently the mosaic virus is less intimately related to the phloem than is the curly top virus, which constantly and primarily induces phloem degeneration in the affected plants.

The mottled mesophyll of the mosaic-diseased plants shows the usual characteristics of mesophyll of mosaic leaves. The yellow areas tend to be thinner than the green areas and show certain juvenile characteristics: their cells are nearly isodiametric and rather closely packed, with no typical differentiation into palisade and spongy layers. Early in development the yellow areas show scanty cell divisions, so that at first they have larger cells and appear more mature than the small-celled green areas in which divisions are still continuing. The green areas resemble healthy mesophyll or appear hyperplastic. The degree of difference between the yellow and the green areas, on the one hand, and between the diseased and healthy leaves, on the other, varies considerably.

The chloroplasts of the yellow areas undergo pronounced pathologic changes. In young cells they are deficient in number, possibly because of delayed development or destruction of plastid primordia. In older cells the chloroplasts are pale and fragile; they readily respond with swelling and disintegration to the contact with tap water. Or the chloroplasts are diffuse and irregularly shaped, and stain lightly. In the most severely diseased cells the chloroplasts fuse into amorphous masses. Nuclei may be absent from such cells. According to the literature, similar degenerative phenomena occur in plastids exposed to many different injurious agents. Apparently they are symptoms of physiologic disturbances which are caused within the diseased cells by the presence of virus, but which are not necessarily responses to any specific action of the virus.

LITERATURE CITED

(1) Alexandrov, W. G.
1929. ZUSTAND UND TÄTIGKEIT DER CHLOROPLASTEN BEI VERSCHIEDENEN KLIMATISCHEN BEDINGUNGEN. Protoplasma 6: [429]–437.

(2) Beauverie, J.
1928. QUELQUES ASPECTS DE LA DÉGÉNÉRESCENCE DES PLASTES, APPLICATIONS AU PARASITISME. Rev. Gén. Bot. 40: 206–225, 264–276, illus.

(3) ———
1938. ÉTUDES DE CYTOLOGIE EXPÉRIMENTALE: ACTION DE LA SAPONINE, DE LA LÉCITHINE ET DE L'OLÉATE DE SODIUM SUR LES CHROMOPLASTES DES PÉTALES DE RENONCULES. Rev. de Cytol. et de Cytophysiol. Vég. 3: [65]–79, illus.

(4) ———
1938. LA STRUCTURE GRANULAIRE DES GRAINS DE CHLOROPHYLLE. Rev. de Cytol. et de Cytophysiol. Vég. 3: [80]–109, illus.

(5) Bennett, C. W.
1940. THE RELATION OF VIRUSES TO PLANT TISSUES. Bot. Rev. 6: 427–473.

(6) BIEBL, R.
1935. DIE WIRKUNG DER α-BESTRAHLUNG AUF PROTOPLASMA UND CHLOROPLASTEN. Protoplasma 24: [225]-257, illus.

(7) BÖNING, K.
1927. DIE MOSAIKKRANKHEIT DER RÜBE. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 3: [81]-128, illus.

(8) ———
1927. DIE MOSAIKKRANKHEIT DER ACKERBOHNE (VICIA FABA L.). Ein Beitrag zu dem Mosaik der Papilionaceen. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 4: [43]-111, illus.

(9) CLINCH, P.
1932. CYTOLOGICAL STUDIES OF POTATO PLANTS AFFECTED WITH CERTAIN VIRUS DISEASES. Roy. Dublin Soc., Sci. Proc. 20: 143-172, illus.

(10) COOK, M. T.
1925. HISTOLOGY AND CYTOLOGY OF SUGAR CANE MOSAIC. Porto Rico Dept. Agr. Jour. 9: 5-27, illus.

(11) ———
1926. THE EFFECT OF MOSAIC ON THE CONTENT OF THE PLANT CELL. Porto Rico Dept. Agr. Jour. 10: 229-238, illus.

(12) ———
1930. THE EFFECT OF SOME MOSAIC DISEASES ON CELL STRUCTURE AND ON THE CHLOROPLASTS. Porto Rico Dept. Agr. Jour. 14: 69-101, illus.

(13) ———
1931. THE EFFECT OF MOSAIC ON CELL STRUCTURE AND CHLOROPLASTS. Porto Rico Depts. Agr. Jour. 15: 177-181, illus.

(14) DICKSON, B. T.
1922. STUDIES CONCERNING MOSAIC DISEASES. Macdonald Col., McGill Univ Tech. Bul 2: 1-125, illus.

(15) DOOLITTLE, S. P.
1920. THE MOSAIC DISEASE OF CUCURBITS. U. S. Dept. Agr. Bul. 879, 69 pp., illus.

(16) DUNLAP, A. A.
1931. CARBOHYDRATE VARIATIONS ACCOMPANYING THE MOSAIC DISEASE OF TOBACCO. Amer. Jour. Bot. 18: 328-336.

(17) ECKERSON, S. H.
1926. AN ORGANISM OF TOMATO MOSAIC. Bot. Gaz. 81: 204-209, illus.

(18) ESAU, K.
1933. PATHOLOGIC CHANGES IN THE ANATOMY OF LEAVES OF THE SUGAR BEET, BETA VULGARIS L. AFFECTED BY CURLY TOP. Phytopathology 23: 679-712, illus.

(19) ———
1934. ONTOGENY OF PHLOEM IN THE SUGAR BEET (BETA VULGARIS L.) Amer. Jour. Bot. 21: 632-644, illus.

(20) ———
1935. ONTOGENY OF THE PHLOEM IN SUGAR BEETS AFFECTED BY THE CURLY-TOP DISEASE. Amer. Jour. Bot. 22: 149-163, illus.

(21) ———
1938. SOME ANATOMICAL ASPECTS OF PLANT VIRUS DISEASE PROBLEMS. Bot. Rev. 4: 548-579.

(22) ———
1941. PHLOEM ANATOMY OF TOBACCO AFFECTED WITH CURLY TOP AND MOSAIC. Hilgardia 13: 437-490, illus.

(23) FAULL, A. F.
1935. ELAIOPLASTS IN IRIS: A MORPHOLOGICAL STUDY. Arnold Arboretum Jour. 16: 225-267, illus.

(24) GICKLHORN, J.
1932. VORÜBERGEHENDE FORMÄNDERUNGEN VON PLASTIDEN WÄHREND DER PLASMOLYSE. Protoplasma 15: [71]-89, illus.

(25) GOLDSTEIN, B.
1926. A CYTOLOGICAL STUDY OF THE LEAVES AND GROWING POINTS OF HEALTHY AND MOSAIC DISEASED TOBACCO PLANTS. Torrey Bot. Club Bul. 53: 499-599, illus.

(26) GRAINGER, J.
1933. THE MOVEMENT OF TOBACCO MOSAIC VIRUS IN ITS HOST. Ann. Appl. Biol. 20: 236–257, illus.

(27) ——— and Heafford, R. M.
1933. SOME EFFECTS OF THE ORDINARY TOBACCO MOSAIC UPON THE DEVELOPMENTAL ANATOMY OF THE HOST PLANT. Leeds Phil. and Lit. Soc. Proc. 2: 406–415, illus.

(28) GRATZY-WARDENGG, E.
1931. DEGENERATION VON CHLOROPLASTEN AN FARNPROTHALLIEN. Protoplasma 14: [52]–63, illus.

(29) GUILLIERMOND, A., MANGENOT, G., and PLANTEFOL, L.
1933. TRAITÉ DE CYTOLOGIE VÉGÉTALE. 1195 pp., illus. Paris.

(30) HEIN, I.
1926. CHANGES IN PLASTIDS IN VARIEGATED PLANTS. Torrey Bot. Club Bul. 53: 411–418, illus.

(31) HEITZ, E.
1936. UNTERSUCHUNGEN ÜBER DEN BAU DER PLASTIDEN. I. DIE GERICHTETEN CHLOROPHYLLSCHEIBEN DER CHLOROPLASTEN. Planta 26: [134]–163, illus.

(32) HOGGAN, I. A.
1927. CYTOLOGICAL STUDIES ON VIRUS DISEASES OF SOLANACEOUS PLANTS. Jour. Agr. Res. 35: 651–671, illus.

(33) HOLMES, F. O.
1928. ACCURACY IN QUANTITATIVE WORK WITH TOBACCO MOSAIC VIRUS. Bot. Gaz. 86: 66–81, illus.

(34) IWANOWSKI, D.
1903. ÜBER DIE MOSAIKKRANKHEIT DER TABAKSPFLANZE. Ztschr. f. Pflanzenkrank. 13: 1–41, illus.

(35) JOHANSEN, D. A.
1940. PLANT MICROTECHNIQUE. 523 pp., illus. New York and London.

(36) KNUDSON, L.
1936. HAS THE CHLOROPLAST A SEMIPERMEABLE MEMBRANE? (Abstract.) Amer. Jour. Bot. 23: 694.

(37) ———
1940. PERMANENT CHANGES OF CHLOROPLASTS INDUCED BY X RAYS IN THE GAMETOPHYTE OF POLYPODIUM AUREUM. Bot. Gaz. 101: 721–758, illus.

(38) KÜSTER, E.
1927. BEITRÄGE ZUR PHYSIOLOGIE UND PATHOLOGIE DER CHLOROPLASTEN. Protoplasma 2: [65]–79, illus.

(39) ———
1937. PATHOLOGIE DER PFLANZENZELLE. II. PATHOLOGIE DER PLASTIDEN. 152 pp., illus. Berlin.

(40) LEE, A. B.
1924. THE MICROTOMIST'S VADE-MECUM. A HANDBOOK OF THE METHODS OF MICROSCOPIC ANATOMY. Ed. 8, 594 pp., illus. Philadelphia. (Reprint.)

(41) LIEBALDT, E.
1913. ÜBER DIE WIRKUNG WÄSSERIGER LÖSUNGEN OBERFLÄCHENAKTIVER SUBSTANZEN AUF DIE CHLOROPHYLLKÖRNER. Ztschr. f. Bot. 5: [65]–113, illus.

(42) MATSUMOTO, T.
1922. SOME EXPERIMENTS WITH AZUKI-BEAN MOSAIC. Phytopathology 12: [295]–297, illus.

(43) MURAVIOV, V. P., ed.
1930. MOSAIC DISEASES OF THE SUGAR BEET. (Mozaichnye bolezni sakharnoi svekly.) U. S. S. R. Soiuzsakhara (Plant Breeding Dept., Union Sugar Concern) 286 pp., illus. Kiev.

(44) NELSON, R.
1932. INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN (PHASEOLUS VULGARIS L.). Mich. Agr. Expt. Sta. Tech. Bul. 118, 71 pp. illus.

(45) PETELER, K.
1939. AMÖBOIDE FORMVERÄNDERUNGEN DER DIATOMEEN-PLASTIDEN. Protoplasma 32: [9]–19, illus.

(46) ROBBINS, W. W.
1921. MOSAIC DISEASE OF SUGAR BEETS. Phytopathology 11: [349]–365, illus.

(47) Roland, G.
1936. RECHERCHES SUR LA JAUNISSE DE LA BETTERAVE ET QUELQUES OBSERVATIONS SUR LA MOSAÏQUE DE CETTE PLANTE. Sucr. Belge 55: 263–268, illus.

(48) Schaffnit, E., and Weber, H.
1927. ÜBER DAS VORKOMMEN VON INTRAZELLULAREN KÖRPERN IN DEN GEWEBEN MOSAIKKRANKER RÜBEN. Forsch. auf dem Geb. Pflanzenkrank. u. Immunität im Pflanzenr. 4: [23]–42, illus.

(49) Schürhoff, P. N.
1924. DIE PLASTIDEN. *In* Linsbauer, K. Handbuch der Pflanzenanatomie. Allgemeiner Teil: Cytologie. Bd. 1, 224 pp., illus. Berlin.

(50) Schwarz, W.
1928. ZUR ÄTIOLOGIE DER GEADERTEN PANASCHIERUNG. (I. MITTEILUNG.) Planta 5: [660]–680, illus.

(51) ———
1930. ÜBER DIE URSACHEN UND DAS ZUSTANDEKOMMEN DER PANASCHIERUNG BEI EINER FORM DER SELAGINELLA MARTENSII SPRING. FOL. VAR. Protoplasma 10: [427]–451, illus.

(52) Senn, G.
1908. DIE GESTALTS- UND LAGEVERÄNDERUNG DER PFLANZEN-CHROMATOPHOREN. 397 pp., illus. Leipzig.

(53) Sheffield, F. M. L.
1933. THE DEVELOPMENT OF ASSIMILATORY TISSUE IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO. Ann. Appl. Biol. 20: 57–69, illus.

(54) Sirotina, M.
1932. A CYTOLOGICAL STUDY OF MOSAIC DISEASE OF SUGAR BEET. (TSITOLOGICHESKOE IZUCHENIE MOZAIKI SAKHARNOI SVEKLY.) Nauk. Zapiski Tsukr. Prom. Ukrain. Nauk.-Dosl. Inst. Tsukr. Prom. [Sci. Contr. Sugar Ind. Ukrain. Res. Inst. Sugar Ind.] 9 (24): 195–216. Illus. [In Russian. English summary.]

(55) Smith, F. F.
1926. SOME CYTOLOGICAL AND PHYSIOLOGICAL STUDIES OF MOSAIC DISEASES AND LEAF VARIEGATIONS. Mo. Bot. Gard. Ann. 13: 425–484. Illus.

(56) Sorokin, H.
1927. PHENOMENA ASSOCIATED WITH THE DESTRUCTION OF THE CHLOROPLASTS IN TOMATO MOSAIC. Phytopathology 17: 363–379, illus.

(57) Stone, W. E.
1942. EFFECTS OF SOME MILD FORMS OF MOSAIC ON POTATO AND A FEW OTHER PLANTS. Jour. Agr. Res. 65: 195–207, illus.

(58) Townsend, C. O.
1915. SUGAR-BEET MOSAIC. Science 42: 219–220.

(59) Weier, T. E.
1933. NEUTRAL RED STAINING IN THE PROTONEMA OF POLYTRICHUM COMMUNE. Amer. Jour. Bot. 20: 431–439, illus.

(60) Weier, [T.] E.
1936. THE STRUCTURE OF THE NON-STARCH-CONTAINING BEET CHLOROPLAST. Amer. Jour. Bot. 23: 645–652, illus.

(61) ———
1938. THE STRUCTURE OF THE CHLOROPLAST. Bot. Rev. 4: 497–530, illus.

(62) ———
1938. VIABILITY OF CELLS CONTAINING CHLOROPLASTS WITH AN OPTICALLY HOMOGENEOUS OR GRANULAR STRUCTURE. Protoplasma 31: [346]–350.

(63) ———
1939. THE MICROSCOPIC APPEARANCE OF THE CHLOROPLAST. Protoplasma 32: [145]–152.

(64) Wieler, A.
1936. ÜBER DEN BAU DER CHLOROPHYLLKÖRNER. Protoplasma 26: [295]–311, illus.

(65) Woods, A. F.
1900. INHIBITING ACTION OF OXIDASE UPON DIASTASE. Science 11: 17–19.

(66) ———
1902. OBSERVATIONS ON THE MOSAIC DISEASE OF TOBACCO. U. S. Bur. Plant Indus. Bul. 18, 24 pp., illus.

REACTION OF LACTUCA SPECIES TO THE ASTER YELLOWS VIRUS UNDER FIELD CONDITIONS [1]

By Ross C. Thompson

Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The loss to lettuce growers attributable to the aster yellows virus in certain localities in the eastern part of the United States has amounted to as much as 30 percent of the crop in some seasons. The increasing seriousness of this disease has emphasized the need for a more satisfactory means of control than is at present available. In the hope of determining whether genetic factors for resistance to the aster yellows virus that might be of value in breeding resistance into the important commercial varieties could be located in any of the numerous species and varieties of lettuce, investigations were initiated. In 1935 a large collection of varieties of cultivated lettuce from both domestic and foreign sources was started at the Bureau of Plant Industry Station, Beltsville, Md. These were tested under field conditions to determine their reaction to the aster yellows virus. After several years of field testing it was concluded that none of the varieties of cultivated lettuce carry factors that are likely to be of value in breeding for resistance to this disease. Attention was then turned to the wild species of *Lactuca*. As many wild forms as could be obtained were collected and tested under field conditions for their reaction to the aster yellows virus.

Yellows of aster (*Callistephus chinensis* (L.) Nees) was described by Smith (*7*),[2] who suggested that similar symptoms in other Compositae might be due to the same cause. According to Kunkel (*2, 3*), the aster yellows virus is spread from plant to plant almost entirely by a single species of leafhopper (*Macrosteles divisus* (Uhl.)). He listed some 170 species of plants to which the virus was transmitted by *M. divisus*. Numerous other host plants have been added to the list. Some of these are wild forms that provide overwintering hosts for the leafhopper; from them the virus is spread to cultivated plants.

Probably the first report of aster yellows in lettuce was made by Carpenter (*1*), who indicated it to be the cause of considerable loss to lettuce growers in the Rio Grande Valley of Texas. Severin (*5*) presented data which indicated that the strain of aster yellows virus found in California differed from the eastern strain. He (*6*) found a number of varieties of lettuce to be susceptible to the California strain. Linn (*4*) listed 23 varieties of lettuce that were susceptible to the eastern strain on Staten Island, N. Y. It is assumed that

---

[1] Received for publication March 10, 1943. The writer is indebted to the Division of Plant Exploration and Introduction for many of the species, varieties, and strains of *Lactuca* used in these investigations.

[2] Italic numbers in parentheses refer to Literature Cited, p. 124.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 3
Aug. 1, 1944
Key No. G-1302

the eastern strain of the aster yellows virus described by the abovementioned workers is the one involved in the study of *Lactuca* reported herein, but no effort has been made to verify the assumption.

MATERIALS

AMERICAN GARDEN VARIETIES

The following varieties of *Lactuca sativa* L. from American sources were grown over a period of several years, and notes were made on their reaction to aster yellows: Asparagus, Batavian Bath Cos, Bibb, Big Boston, Black-Seeded Simpson, Brown Batavian, California Cream Butter, Chavigne, Crisp as Ice, Dark Green Cos, Deacon, Deer's Tongue, Density, Denver Market, Early Curled Simpson, Giant Summer, Golden Queen, Grand Rapids, Green Madeira Winter, Green Province Cos, Hanson, Hardy Green Winter, Hardy Red Winter, Hubbards Market, Iceberg, Imperial C, Imperial D, Imperial F, Imperial 44, Imperial 152, Imperial 615, Imperial 847, Imperial 850, Malta, Mammoth Black-Seeded Butter, Marvel, Maximum, May King, Mignonette, Milly, New York Regular, New York 12, New York 515, Paris White Cos, Prize Head, Salamander, Speckled Dutch Butter, Tennisball, Tom Thumb, Unrivalled, and Wayahead.

EUROPEAN GARDEN VARIETIES

The following European varieties of lettuce obtained from Vilmorin-Andrieux & Cie., Paris, France, were included among the varieties studied: Batavian Bath, Brown Batavian, Brown Genoa, Brown Stolid, Brown Stonehead, Brown Winter, Giant Summer, Green Madeira Winter, Green Province Cos, Hardy Green Winter, Hardy Red Winter, Madeira Large Winter, Market Gardener's Red, Marvel, Milly, Red Edged Victoria, and White Batavian.

INTRODUCTIONS

In addition to the varieties just listed a large number of strains of *Lactuca* obtained from various foreign sources through the Division of Plant Exploration and Introduction have been grown and their reactions to the aster yellows virus have been noted. These strains had the following P. I. numbers: 113351 to 113353, 114033, 115189 to 115191, 115476, 115591, 115592, 115943, 116517, 116933, 117208 to 117216, 117892 to 117895, 118306, 118307, 118309 to 118317, 118319 to 118321, 118601, 118683, 118684, 119773, 120932 to 120938, 120940 to 120949, 120951 to 120966, 121935 to 121938, 121940, 124124, 127335, 131425, 133493, 133494, 134205, 134206, 138584 to 138598, 138912, 140392 to 140402, 141680 to 141683, 141961, 141962, 142863 to 142872. Many of these introduced strains were obviously American and European named varieties or very similar strains, but no attempt was made to identify them.

WILD SPECIES

The following *Lactuca* species obtained from the sources indicated were included in the tests in 1939, 1940, 1941, and 1942:

Lactuca altaica Fisch. and Mey., from Egypt, through Division of Plant Exploration and Introduction.

L. bourgaei (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.

L. canadensis L., collected in Maryland.

L. floridana (L.) Gaertn., collected in Maryland.

L. graminifolia Michx., from South Carolina, through the late J. B. Norton.

L. indica L., from China, through G. L. Stebbins, Jr.

L. marschallii Stebbins, from Sweden, through G. L. Stebbins, Jr.

L. muralis (L.) Fresen., from Sweden, through G. L. Stebbins, Jr.

L. perennis L., from Wayside Gardens, Mentor, Ohio.

L. raddeana Maxim., from Union of Soviet Sociali t Republics, through Division of Plant Exploration and Introduction.

L. saligna L., from Ohio, through G. L. Stebbins, Jr.

L. saligna L. (P. I. No. 120950), from Turkey, through Division of Plant Exploration and Introduction.

L. serriola L., collected in Rock Creek Park, D. C.

L. serriola L., (P. I. No. 114512), from Sweden, through Division of Plant Exploration and Introduction.

L. serriola L. (P. I. No. 114535), from Afghanistan, through Division of Plant Exploration and Introduction.

L. serriola L. (P. I. No. 125819), from Afghanistan, through the Division of Plant Exploration and Introduction.

L. spicata (Lam.) Hitchc., collected in Virginia.

L. squarrosa (Thunb.) Miq., from Union of Soviet Socialist Republics, through Division of Plant Exploration and Introduction.

L. squarrosa (Thunb.) Miq. (*L. laciniata* (Houtt.) Makino), from Asia, through Division of Plant Exploration and Introduction.

L. tatarica (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.

L. virosa L. (annual form), from Surrey, England, through Division of Plant Exploration and Introduction.

L. virosa L. (biennial form), from Stockholm, Sweden, through Division of Plant Exploration and Introduction.

METHODS

The tests for response to the aster yellows virus were all made under field conditions. The seed was planted in wooden flats in a greenhouse, and when the seedlings were large enough to transplant they were removed from the flats and set in 5-ounce paper drinking cups. The plants were later transplanted to the field where they were exposed to natural infection. The large number of strains to be tested made it impractical to attempt controlled inoculations.

Many of the varieties of cultivated lettuce were grown for only one season. If a variety showed a fairly large number of yellows-infected plants, it was considered susceptible and probably of no value for breeding for resistance and was eliminated from future tests. If the number of infected plants was quite small in the first planting, the variety was grown again before eliminating it.

RESULTS

The loss from aster yellows in spring lettuce has always been very high in plantings at the Bureau of Plant Industry Station, Beltsville, Md. Even in years when the percentage of affected plants has been lowest, field tests for varietal response to the virus have been satisfactory. A high percentage of the plants of susceptible varieties have always been diseased when the crop has been started early.

CULTIVATED VARIETIES

It was at first thought that some of the plants of the cultivated varieties that did not develop yellows might be resistant. Many such plants have been tested for resistance in succeeding generations, but in no case have they shown indication of resistance. In 1937, a year of high field infection, approximately 10,000 plants of many cultivated varieties and strains were checked throughout the season for plants that escaped infection. Many of the plants were lost during the summer from causes other than aster yellows. At the end of the season (late August) only 1 plant that did not show infection with yellows survived to produce seed. Seed of this plant was saved, and 200 plants were grown in the field the following year. Some of these escaped the yellows, but none of them showed any indication of resistance in later generations.

Because of the great number of leafhoppers present in the fields where these tests were conducted, it has often been difficult to see how any plants could escape inoculation. As the season advanced the number of infected plants increased, greatly magnifying the chances for the spread of the virus. However, numerous subsequent tests of disease-free plants of the cultivated varieties indicate that they escaped inoculation rather than that they were resistant.

That the yellows-free individuals are plants that have somehow escaped infection and are not resistant is as would be expected, since natural crossing in lettuce is limited and most varieties are highly homozygous. For this reason, it seems fairly safe to assume that the appearance of even a small percentage of diseased plants in a population is sufficient to permit the variety to be classed as susceptible. Single 50-foot rows of many varieties were enough to show their susceptibility; however, as many as 200 plants of some varieties were grown.

All 200 lots of cultivated lettuce obtained from American and European seedsmen and from various foreign sources, through the Division of Plant Exploration and Introduction, were found to be susceptible to the aster yellows virus. Although the varieties tested do not exhaust the list of known varieties and strains of cultivated lettuce, the number of strains included makes doubtful the finding of genetic factors for resistance to the aster yellows virus in *Lactuca sativa*.

WILD SPECIES

Preliminary tests of wild species indicated that some of them were much more resistant to the virus than the cultivated varieties. A replicated plot test was set up with the intention of growing 4 replicated plots of 25 plants of each species. Such tests were carried out over the 4-year period, 1939 to 1942. A few species were added to the list after the tests were started; hence they were not grown all 4 years. Some of the species were found to be especially susceptible to diseases other than aster yellows. In some cases an entire replication of certain species was lost through attacks of some of these diseases. Heavy rains in some seasons and severe drought in others resulted in damage to and loss of plants. As a consequence of these and other factors there were great differences both in the number of plants per plot and in the number of plants of different species.

In the meantime hybridization studies in which the genetic compatibility of many of these wild species with *Lactuca sativa* was investigated were reported by Thompson (*8*) and Thompson, Whitaker, and Kosar (9). The studies indicated that, because of genetic incompatibility, many of the wild species could be of no value for breeding even though they might be highly resistant to aster yellows.

The results from the field tests of wild species are given in table 1 in terms of percentage of plants showing symptoms of aster yellows.

TABLE 1.—*Summary of data on reaction of some species of Lactuca to the aster yellows virus*

Species of *Lactuca*	1939		1940		1941		1942		Total	
	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows	Plants tested	Plants with yellows
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
L. altaica	45	42.22	93	22.58	99	11.11	76	11.84	313	19.17
L. bourgaei	62	0	81	0	83	0	35	0	261	0
L. canadensis	93	7.53	76	5.26	91	6.79	83	6.02	343	6.71
L. floridana	92	8.70	87	5.75	97	13.4	89	7.87	365	9.04
L. graminifolia	95	6.32	93	2.15	95	12.63	73	6.85	356	7.02
L. indica	85	34.12	98	7.14	83	20,48	79	16.46	345	19.13
L. marschallii	......	......	75	0	50	0	99	0	224	0
L. muralis	46	76.10	93	13.98	77	32.47	61	31.15	277	33.21
L. perennis	79	49.37	90	6.67	73	20.55	85	8.24	327	20.49
L. raddeana	87	5.75	95	3.16	71	9.86	95	8.42	348	6.61
L. saligna (*native*)	99	9.09	94	8.51	99	7.07	87	4.60	379	7.39
L. saligna (P. I. No. 120950)	100	5.00	91	4.40	94	2.13	91	3.30	376	3.72
L. serriola (local)	......	......	86	5.81	65	3.08	15	6.67	166	4.82
L. serriola (P. I. No. 114512)	......	......	......	......	88	5.68	88	1.14	176	3.41
L. serriola (P. I. No. 114535)	98	29.59	97	8.24	91	27.47	90	12.22	376	19.41
L. serriola (P. I. No. 125819)	86	59.30	99	17.17	97	27.84	88	28.41	370	32.43
L. spicata	99	18.18	92	6.52	87	17.24	86	5.81	364	12.09
L. squarrosa	......	......	87	17.24	90	16,67	......	......	177	16.95
L. squarrosa (*L. laciniata*)	89	98.87	96	36.46	92	64.13	95	49.47	372	61.56
L. tatarica	90	0	78	0	81	0	91	0	340	0
L. virosa (annual)	99	79.80	89	48.31	93	61,29	97	61.86	378	63.23
L. virosa (biennial)	......	......	95	31.58	91	60.44	87	48.88	273	46.52

Since the loss of plants from various causes introduced great variation in the field plot data and all but two of the species have been eliminated as possible breeding material because of their genetic incompatibility with *Lactuca sativa*, no attempt has been made to use the data to indicate the relative significance of the differences in the response of the species to the aster yellows virus.

Three of the 22 species and strains of wild lettuce included in these tests (*Lactuca bourgaei*, *L. marschallii*, and *L. tatarica*) seemed immune to the virus. No plants of any of these 3 species showed symptoms of yellows in any of the replications in any of the years in which they were tested. Thompson, Whitaker, and Kosar (*9*) and Thompson (*8*) found that these 3 species were genetically incompatible with *Lactuca sativa*; hence, they are of no direct value in breeding for yellows resistance in cultivated lettuce.

Four 17-chromosome species (*Lactuca canadensis*, *L. floridana*, *L. graminifolia*, and *L. spicata*) showed comparatively low percentages of diseased plants. These 4 have also been found by the above-named writers to be genetically incompatible with *L. sativa*; hence, they are of no direct value as breeding material. The appearance of yellows in some of these species is not proof of complete susceptibility as is the presence of diseased plants in cultivated varieties, since some of the former are not entirely homozygous and some degree of resistance may be present in some plants even though many plants succumb to the virus.

Lactuca squarrosa (*L. laciniata*) and *L. virosa* had consistently high percentages of diseased plants and are apparently very susceptible to the virus. These two species have also been found to be genetically incompatible with *L. sativa.*

Of the 22 species and strains tested only the strains of *Lactuca serriola* and *L. saligna* indicate value as breeding material and there is some question whether any use can be made of *L. saligna*. The data indicate that 2 strains of *L. serriola,* the local strain and P. I. No. 114512, and the 2 strains of *L. saligna* may be of value in breeding for aster yellows resistance in *L. sativa*. The *L. serriola* strains cross readily with *L. sativa* and give fertile progenies. So far it has not been possible to cross the *L. saligna* strains with *L. sativa*. The *L. saligna* strains have both been crossed with *L. serriola,* and partly fertile hybrids have resulted. It may be possible to use the *L. saligna* strains in breeding by transferring the desired hereditary factors to *L. serriola* and then through the hybrid to *L. sativa.* So far the attempt to make a cross between the *L. serriola* × *L. saligna* hybrid and *L. sativa* has not been successful. An important point not yet investigated is whether the low disease incidence of these *L. serriola* and *L. saligna* strains is due to direct resistance to the virus or to some peculiarity of the plant that repels the vector. The consistently low percentages of diseased plants occurring in these 4 strains indicate that they have some capacity for resisting virus infection and may be of value in breeding for resistance to the aster yellows virus.

SUMMARY

Two hundred varieties and strains of cultivated lettuce and 22 wild species and strains of *Lactuca* were tested under field conditions for their reaction to the aster yellows virus. All the strains of the cultivated varieties indicated susceptibility to the virus. The wild species varied greatly in their susceptibility to infection under field conditions.

Lactuca tatarica, L. bourgaei, and *L. marschallii* seemed to be immune to the aster yellows virus, but they have no direct value in breeding for resistance, since they have been found to be genetically incompatible with the cultivated varieties.

Two strains of *Lactuca serriola* and two strains of *L. saligna* showed marked ability to escape infection. These species may prove to be of value in breeding for resistance in cultivated lettuce; especially the *L. serriola* forms, which cross readily with cultivated lettuce.

The 17-chromosome species (*Lactuca canadensis, L. spicata, L. graminifolia, and L. floridana*) as a group indicated some ability to escape infection, but they offer no promise as material for breeding for aster yellows resistance, as they have been found to be genetically incompatible with the cultivated form.

Lactuca virosa and *L. squarrosa* (*L. laciniata*) are highly susceptible as was indicated by a consistently high percentage of infected plants.

LITERATURE CITED

(1) Carpenter, C. W.
1916. THE RIO GRANDE DISEASE OF LETTUCE. Phytopathology 6: 303–305, illus.

(2) Kunkel, L. O.
1926. STUDIES ON ASTER YELLOWS. Amer. Jour. Bot. 13: 646–705, illus.

(3) KUNKEL, L. O.
1931. STUDIES ON ASTER YELLOWS IN SOME NEW HOST PLANTS. Boyce Thompson Inst. Contrib. 3: 85–123, illus.

(4) LINN, M. B.
1940. THE YELLOWS DISEASE OF LETTUCE AND ENDIVE. N. Y. (Cornell) Agr. Expt. Sta. Bul. 742, 33 pp., illus.

(5) SEVERIN, H. H. P.
1934. EXPERIMENTS WITH THE ASTER-YELLOWS VIRUS FROM SEVERAL STATES. Hilgardia 8: 305–325, illus.

(6) ———
1929. YELLOWS DISEASE OF CELERY, LETTUCE, AND OTHER PLANTS TRANSMITTED BY CICADULA SEXNOTATA (FALL.) Hilgardia 3: 543–582, illus.

(7) SMITH, R. E.
1902. GROWING CHINA ASTERS. Mass. (Hatch) Agr. Expt. Sta. Bul. 79, 26 pp., illus.

(8) THOMPSON, R. C.
1943. FURTHER STUDIES ON INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA. Jour. Agr. Res. 66: 41–48, illus.

(9) ——— WHITAKER, T. W., and KOSAR, W. F.
1941. INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA. Jour. Agr. Res. 63: 91–107, illus.

DIFFERENTIAL EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF THE BEET LEAFHOPPER[1, 2]

By F. H. Harries

Associate entomologist, Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The maturation and time of movement of the spring generation of beet leafhoppers (*Eutettix tenellus* (Bak.)) from desert breeding areas to the cultivated sections and the seasonal sequence and number of the generations produced annually are governed largely by temperature. Since it was desirable to know more definitely the direct effect of temperature and changes in temperature on development of the insect during investigation of these and other problems of seasonal development, maturation of the different stages of the leafhopper was studied under controlled conditions.

Investigations by various workers (*6,8,9,10,13*)[3] have indicated that insect development may occur more rapidly at varying temperatures than would be expected from results obtained at constant temperatures. This effect has usually been attributed to a stimulus produced by temperature changes or, conversely, to an inhibitory influence of the unnatural exposure to the same temperature during the entire period of development. With few if any exceptions, effects of temperature on the rate of insect development have not been shown directly, but have been evaluated on the basis of end results indicated by index values taken as reciprocals of the time required to complete definite stages of metamorphosis. In this method it is assumed that at different temperatures the same portions of development require equal fractions of the total time necessary for completion of development.

There is evidence, however, that two or more growth phases unequally affected by different temperatures may occur within the same stage of development. This was shown by Crozier (*8*) from data by Titschack (*15*) on the pupal period of *Tineola bisselliella* (Hum.) and by Bliss (*2*) on the prepupa of *Drosophila melanogaster* Meig. Brown (*5*), analyzing the same data in conjunction with further studies on the development of a cladoceran at different temperatures, concluded that, in accordance with the nature of the equation proposed by Crozier, the analytical expression used to describe growth must have at least two velocity constants unequally affected by different temperatures. Powsner (*14*) showed that the temperature coefficient differed in successive portions of the embryonic period of *D. melanogaster*, and other data on the pupal stage of the same insect by Ludwig and Cable (*11*) support this interpretation.

---

[1] Received for publication April 22, 1943.

[2] The writer is indebted to J. R. Douglass and W. C. Cook for supervision during the present study and to P. N. Annand for other assistance and suggestions.

[3] Italic numbers in parentheses refer to Literature Cited, p. 135.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 3
Aug. 1, 1944
Key No. K-332

When eggs of the beet leafhopper were exposed alternately for 12-hour periods to two temperatures, development was always completed in less time than would be expected on the basis of the time required for complete development at each constant temperature (*9*). This difference in time represents a stimulating effect of change in temperature provided the course of development is modified solely by the time required for completion of the entire stage. To determine if any factor besides time is involved, the experiments described in this paper were initiated.

METHODS

The eggs and nymphs were obtained from a supply of adults that had been collected in the fall and kept in the laboratory during the winter. Small sugar-beet plants selected for uniformity of size and color were used as hosts. They were grown in the greenhouse in 4-inch pots and were used when about 4 to 6 inches high.

Eggs were obtained by placing gravid females on the plants in small cages designed to fit snugly around the petioles. After 12 hours the cages were removed and the portions of the plant in which the eggs were embedded were marked with ink. Toward the end of embryonic development the cages were again placed over the petioles to confine the young nymphs. In studies of the nymphal stage the insects were confined in cages placed over entire plants. These cages were about 6 inches high and 3 inches in diameter, and were made of thin, fine-meshed cloth over a frame of celluloid ribs. A metal band attached to the frame was pressed into a layer of sand covering the soil around the potted beets. Both types of cages were designed to provide a maximum circulation of air, and thus to minimize any effect of transpiration on temperature and atmospheric moisture within the cages.

The insects were exposed in cabinets[4] in which the temperature was controlled within 1° F. and the relative humidity was kept between 49 and 51 percent. Continuous records were obtained with hygrothermograph recorders, and temperature and humidity within the cages were checked with a thermometer and a small dew-point tube. Insects were subjected to a change in temperature during development by transfer from one chamber to another. Some groups of eggs and nymphs were transferred from a higher to a lower temperature and others from a lower to a higher temperature. Direct observations of the growth of nymphs were made daily by weighing groups of 50 on an analytical balance.

RESULTS AND DISCUSSION

The effect of exposure of the beet leafhopper at one temperature for the first part of the egg or nymphal period and at another temperature for the remainder of the stage is shown in table 1. The duration observed when the temperature was changed is compared with that expected on the basis of the durations observed when the insects were exposed for the entire stage at each of the temperatures involved.

---

[4] ANNAND, P. N., and HARRIES, F. H. TEMPERATURE-HUMIDITY CONTROLLED CABINETS FOR THE STUDY OF INSECTS U. S. Bur. Ent. and Plant Quar. ET-159, 6 pp., illus. 1940. [Processed.]

TABLE 1.—*Duration of the egg and nymphal stages of the beet leafhopper when exposed for different periods to one temperature before being allowed to complete development at another temperature*

EGG STAGE

Temperature° F. First	Temperature° F. Second	Individuals hatched or reared	Duration of exposure to first temperature	Duration of stage: Observed	Duration of stage: Expected	Duration of stage: Difference	Relative difference in duration[1]
		Number	*Days*	*Days*	*Days*	*Days*	*Percent*
90	65	603	0.0	6.27±0.02			
65	90	280	.0	26.35± .06			
90	65	135	1.0	21.63± .08	[2] 23.15	−1.52	−5.8
		110	1.5	20.12± .09	21.54	−1.42	−5.4
		219	2.0	18.12± .06	19.94	−1.82	−6.9
		287	2.5	16.69± .08	18.34	−1.65	−6.3
		280	3.0	14.86± .06	16.74	−1.88	−7.1
		86	3.5	13.17± .07	15.14	−1.97	−7.5
		158	4.0	11.45± .08	13.54	−2.09	−7.9
		177	4.5	9.73± .05	11.94	−2.21	−8.4
		100	5.0	8.24± .09	10.34	−2.10	−8.0
65	90	171	4	9.28± .02	9.32	−.04	−.6
		169	5	10.11± .03	10.08	.03	.5
		244	10	13.61± .03	13.89	−.28	−4.5
		392	12	15.42± .04	15.41	.01	.2
		158	15	17.81± .04	17.70	.11	1.8
		236	20	22.20± .02	21.51	.69	11.0

NYMPHAL STAGE

Temperature° F. First	Temperature° F. Second	Individuals hatched or reared	Duration of exposure to first temperature	Duration of stage: Observed	Duration of stage: Expected	Duration of stage: Difference	Relative difference in duration[1]
90	75	423	0	14.50±0.07			
75	90	463	0	27.50+ .12			
90	75	156	3	25.08± .22	24.81	.27	1.0
		412	6	21.49+ .13	22.12	−.63	−2.3
		486	9	17.87± .13	19.43	−1.56	−5.7
		239	12	16.78± .19	16.74	.04	.1
75	90	226	5	17.51± .08	16.86	.65	4.5
		146	10	20.08± .13	19.23	.85	5.9
		158	15	23.02± .10	21.59	1.43	9.9
		226	20	26.01± .10	23.95	2.06	14.2

[1] Difference in duration of stage expressed as percent of time required for development when exposed to the second temperature only.

[2] Expected duration in this case $=1+\frac{5.27}{6.27}(26.35)$.

When the change was from the higher to the lower temperature, the observed duration of development was usually shorter than the expected duration. Although only one change in temperature was made, some of the data show a decrease in duration of the stage comparable with that produced when temperature changes were made twice daily. On the other hand, when the change was reversed the duration was usually longer than expected. These differences seemed to increase as the duration of exposure at the first temperature increased. Corresponding differences associated with increases and decreases in temperature were not well correlated, but this could probably be expected, since the values are especially subject to experimental error in being dependent on the accuracy of three different means, the interval required for complete development at each of the two constant temperatures, and the interval required when the change was made from one constant temperature to another.

From such results it may be concluded that the relative durations of successive phases of development within the egg and nymphal stages differ at different temperatures. For example, the percentage of growth attained after half the time required for completion of the stage at a given temperature may be more, or less, than that reached after half the time necessary at a different temperature, and a change from one temperature to another may result in apparent differences in developmental time without actually exerting a stimulating or inhibiting effect.

The progress of nymphal development at different temperatures is shown more directly by the daily weights given in table 2. These determinations are averages for a large number of nymphs, but since the insects were weighed in groups of 50, calculation of the standard errors was precluded by the limited number of samples.

TABLE 2.—*Average daily weights of the beet leafhopper during nymphal development at different constant temperatures and 50 percent relative humidity* [1]

Days after hatching	100° F.		90° F.		85° F.		80° F.		75° F.	
	Insects	Average weight	Insects	Average weight	Insects	Average weight	Insects	Average weight	Insects	Average weight
	Number	*Milligram*	*Number*	*Milligram*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*
1	350	0.047	300	0.043	400	0.043	450	0.042	400	0.040
2	350	.060	350	.053	350	.051	400	.051	400	.045
3	350	.084	400	.067	350	.058	500	.055	450	.048
4	350	.101	400	.084	400	.067	450	.065	450	.054
5	350	.157	400	.126	350	.086	400	.078	400	.061
6	350	.202	500	.168	400	.104	350	.100	400	.069
7	350	.280	450	.198	350	.143	400	.123	500	.082
8	350	.389	400	.279	400	.174	400	.153	500	.090
9	350	.481	500	.335	400	.208	600	.189	600	.110
10	350	.589	550	.448	350	.273	350	.228	550	.133
11	350	.701	650	.592	350	.353	450	.256	550	.154
12	350	.800	500	.680	400	.397	450	.319	550	.173
13	350	.828	550	.835	350	.527	350	.342	500	.194
14	140	.932	260	.994	350	.619	450	.417	600	.215
15					300	.740	350	.482	550	.234
16					300	.814	350	.590	550	.279
17					180	1.004	350	.725	650	.313
18							250	.791	550	.364
19							250	.906	550	.412
20							200	1.043	550	.444
21									400	.544
22									450	.622
23									600	.658
24									550	.739
25									500	.868
26									400	.913
27									250	1.064
Average age when becoming adult (days)	13.84		14.5		17.39		20.48		27.48	

[1] The last weight in each column is that of the newly developed adult.

In figure 1 the daily course of growth is shown to define a typical sigmoid curve, in which the growth rate increases during the major part of the period and then decreases as the adult state is approached. The ratio of daily increase in weight, or the relative rate of growth, may be shown by plotting the logarithms of nymphal weight against time, as in figure 2. A linear distribution of the logarithms of successive weights would indicate a constant value for the relative rate of growth. The entire curve for each temperature is not a straight line, however, but appears to consist of linear segments of different slopes, which indicate that growth occurred at different constant rates in successive periods. These segments probably represent the five nymphal instars. With linear measurements of the mouth parts of *Drosophila melanogaster*, Alpatov (*1*) showed that the growth curve consisted of three distinct segments when based on larvae of the same age, and that these periods were delineated as three com-

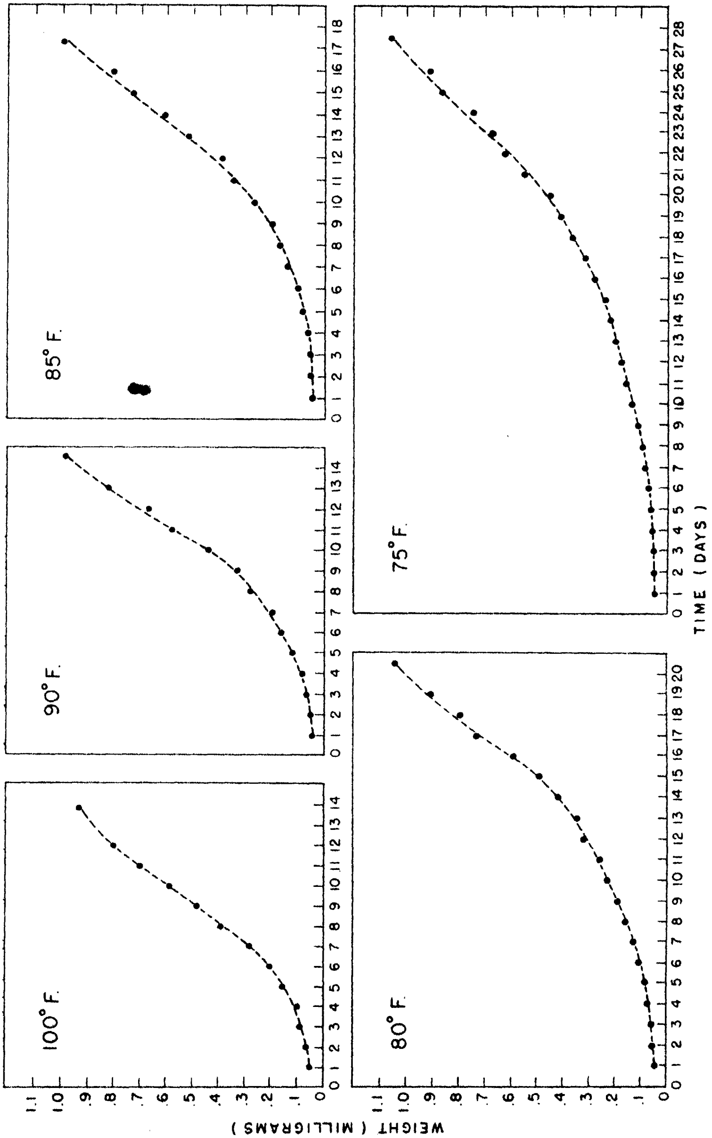


FIGURE 1.—Daily weights of nymphs of the beet leafhopper when reared at constant temperatures.

plete growth cycles when data on the different instars were plotted separately. Similar S-shaped curves were shown by Brindley (*4*) for larval growth of *Ephestia kuehniella* Zell. and *Tribolium confusum*

Duv. at a constant temperature, but the 3-day intervals at which the weight increments were determined were evidently too long to show possible differences in the rate of growth in different instars.

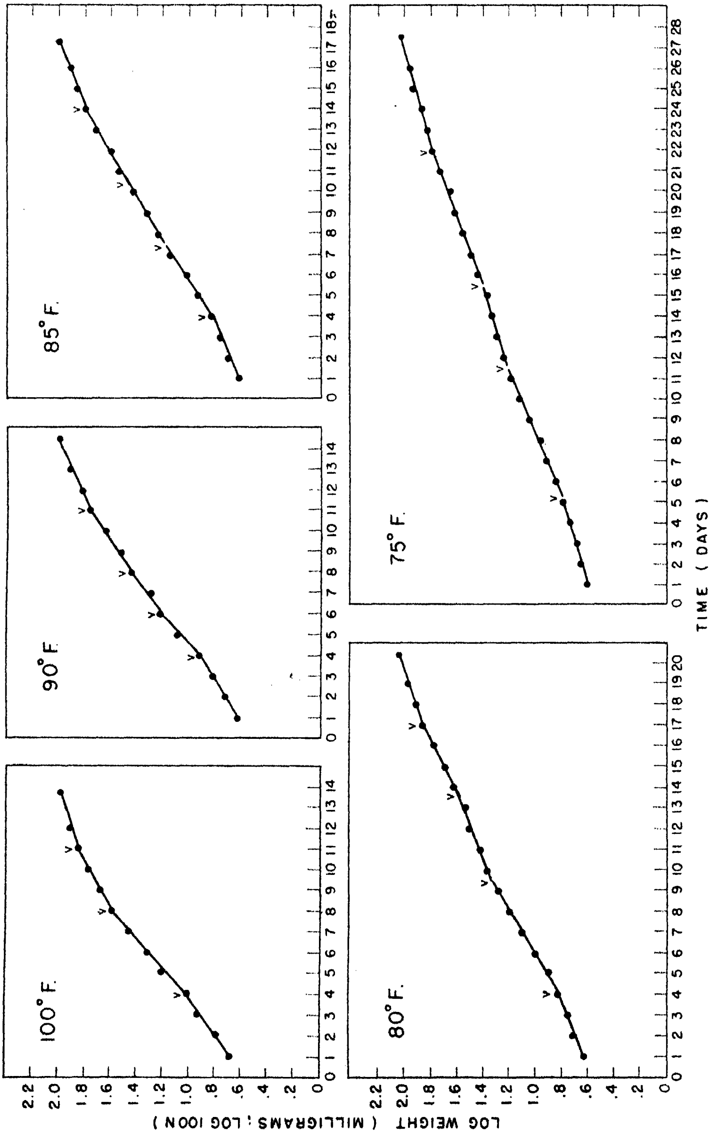


FIGURE 2.—Logarithms of daily weights of nymphs of the beet leafhopper reared at constant temperatures, illustrating changes in the growth ratio as development proceeds. Different curve segments are fitted to the equation $\log y = \log A + bx \log e$ by the method of least squares.

A comparison of the time course of growth at different temperatures is shown in figure 3, where the time factor is expressed in percentage of the total time required for development and the weight factor in percentage of the adult weight. These curves, which are based on figures calculated from the regression lines of figure 2, show distinct divergence in most cases with differences of only 5° F., and the divergence becomes greater with greater differences in temperature. The fact that the curves do not coincide throughout shows that the relative rate of growth at different periods varies with the temperature, as was demonstrated by data in table 1 on the time required for nymphal development in successive exposures to two different temperatures.

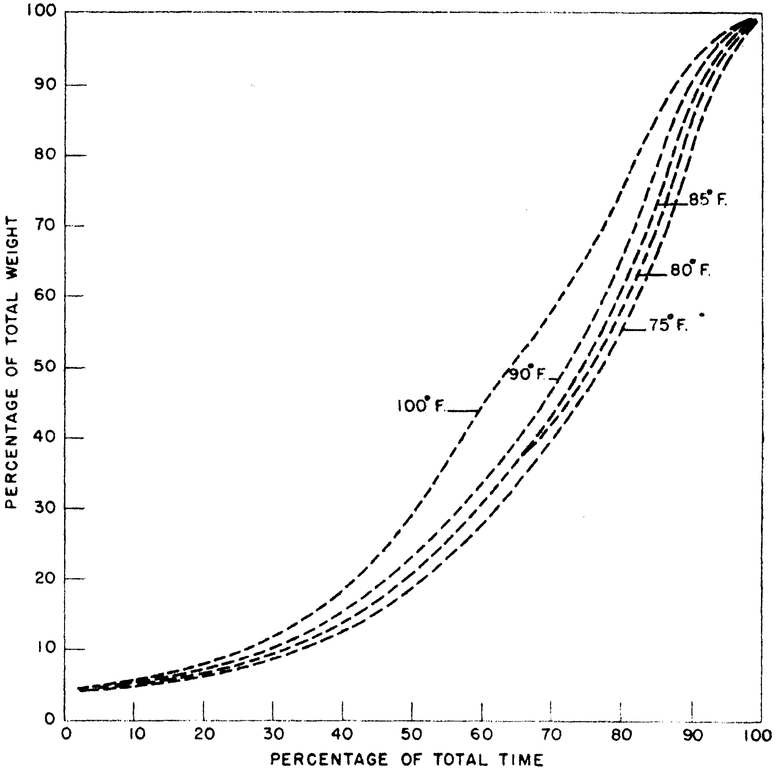


Figure 3.—Nymphal-growth curves at different temperatures, in which percentage of the adult weight is plotted against the percentage of the time required for development.

Regardless of the acceleration produced by changes in temperature, the nature of the growth curves makes it impossible to compute accurately the degree of acceleration from data on the duration of the entire stage at constant temperatures. Crozier (*8*) has shown that part of the difference in time resulting from a change from one temperature to another may be retained following a transfer back to the first temperature. It is uncertain how much of the apparent acceleration due to alternate 12-hour exposures to different temperatures (*9*) may be accounted for in this way, since differences in time resulting from changes in temperature would seem to be rather closely compensating in opposite transfers. In the same way effects of diurnal changes and alternate variations in temperature under natural conditions would tend to be compensating. In view of the effects of successive exposures to two temperatures shown in table 1, the greatest apparent acceleration or deceleration would evidently occur in successive periods of cool and warm weather and during the spring and fall when differences in daily mean temperatures are most pronounced. The relatively more rapid development at lower temperatures in the later nymphal stages would evidently be of considerable advantage to the species in permitting more of the larger nymphs, which occur abundantly late in the fall, to reach the adult stage, in which the insect passes the winter.

Some acceleration in rate of development of the beet leafhopper under alternating temperatures was observed in cases where the lower temperature was apparently well below the minimum for complete development (*9*). These differences in time cannot be explained on the basis of the foregoing discussion since the entire development must be assumed to have occurred at the higher temperature. Exclusive of diapause effects in exposure to cold, as reported by Bodine (*3*), Parker (*12*), Burdick (*6*), and others, evidence of a direct stimulus is shown by the results of exposure of other insects to temperatures below developmental zero, as in studies of Ludwig (*10*), Ludwig and Cable (*11*), and Cook (*7*). Cook found that the metabolism of cutworm larvae was accelerated at higher temperatures after exposure to cold near developmental zero and then gradually declined until a constant rate was reached. This effect was renewed in successive exposures to maintain an accelerated rate at the higher temperature.

The occurrence of successive growth phases that differ only in temperature coefficient may make it extremely difficult to determine the actual amount of stimulus or retardation produced under varying temperatures. As a result of this limitation in conventional methods of analysis based on duration of the stages, other time differences may be shown that are not attributable to a stimulus of temperature changes or, conversely, to an inhibitory influence of unnatural constant temperatures. Although problems of the relation of temperature to insect development are made more difficult, such effects of differences in temperature coefficient are important to an understanding of the influence of environment and to the proper interpretation of experimental results.

SUMMARY

The development of eggs and nymphs of the beet leafhopper (*Eutettix tenellus* (Bak.)) when exposed at one temperature for a given length of time and allowed to complete development at a second temperature was studied in the laboratory. The observed time was compared with that expected on the basis of the time required when the temperature was not changed.

A change from a higher to a lower temperature resulted in completion of development in a shorter time than was expected. This effect was evidently not due entirely to a direct stimulus, since a reverse change between the same two temperatures usually resulted in an increase in the time required rather than a decrease. The differences in time required for development more logically indicated that the difference in growth rate at two temperatures was less in the earlier part of the embryonic and nymphal periods, and greater in the later parts, than was indicated by the average rates based on the time required for completion of the entire stages at each temperature.

Other studies of the time course of growth were made by weighing the nymphs daily during their development at different temperatures. When logarithms of the weights were plotted against time, the curves appear to consist of segments of different slope, which indicate that the growth rate differed in the five nymphal instars. The fact that the curves did not coincide when superimposed by expressing time and rate in percentages corroborated the previous results in showing the presence of two or more phases within the nymphal stage that are unequally affected by different temperatures.

LITERATURE CITED

(1) Alpatov, W. W.
1929. GROWTH AND VARIATION OF THE LARVAE OF DROSOPHILA MELANOGASTER. Jour. Expt. Zool. 52: 407–432, illus.
(2) Bliss, C. I.
1926. TEMPERATURE CHARACTERISTICS FOR PREPUPAL DEVELOPMENT IN DROSOPHILA MELANOGASTER. Jour. Gen. Physiol. 9: 467–495, illus.
(3) Bodine, J. H.
1925. EFFECT OF TEMPERATURE ON RATE OF EMBRYONIC DEVELOPMENT OF CERTAIN ORTHOPTERA. Jour. Expt. Zool. 42: 91–109, illus.
(4) Brindley, T. A.
1930. THE GROWTH AND DEVELOPMENT OF EPHESTIA KUEHNIELLA ZELLER (LEPIDOPTERA) AND TRIBOLIUM CONFUSUM DUVAL (COLEOPTERA) UNDER CONTROLLED CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY. Ent. Soc. Amer. Ann. 23: 741–757, illus.
(5) Brown, L. A.
1927. ON THE NATURE OF THE EQUATION FOR GROWTH PROCESSES. Jour. Gen. Physiol. 11: 37–42, illus.
(6) Burdick, H. C.
1937. THE EFFECTS OF EXPOSURE TO LOW TEMPERATURE ON THE DEVELOPMENTAL TIME OF EMBRYOS OF THE GRASSHOPPER MELANOPLUS DIFFERENTIALIS (ORTHOPTERA). Physiol. Zool. 10: 156–170, illus.
(7) Cook, W. C.
1927. SOME EFFECTS OF ALTERNATING TEMPERATURES ON THE GROWTH AND METABOLISM OF CUTWORM LARVAE. Jour. Econ. Ent. 20: 769–782, illus.
(8) Crozier, W. J.
1926. ON CURVES OF GROWTH, ESPECIALLY IN RELATION TO TEMPERATURE. Jour. Gen. Physiol. 10: 53–73, illus.
(9) Harries, F. H.
1943. SOME EFFECTS OF ALTERNATING TEMPERATURES AND EXPOSURE TO COLD ON EMBRYONIC DEVELOPMENT OF THE BEET LEAFHOPPER. Jour. Econ. Ent. 36: 505–509, illus.
(10) Ludwig, D.
1928. THE EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF AN INSECT (POPILLIA JAPONICA NEWMAN). Physiol. Zool. 1: 358–389, illus.
(11) ——— and Cable, R. M.
1933. THE EFFECT OF ALTERNATING TEMPERATURES ON THE PUPAL DEVELOPMENT OF DROSOPHILA MELANOGASTER MEIGEN. Physiol. Zool. 6: 493–508, illus.

(12) PARKER, J. R.
1930. SOME EFFECTS OF TEMPERATURE AND MOISTURE UPON MELANOPLUS MEXICANUS MEXICANUS SAUSSURE AND CAMNULA PELLUCIDA SCUDDER (ORTHOPTERA). Mont. Agr. Expt. Sta. Bul. 223, 132 pp., illus.
(13) PEAIRS, L. M.
1927. SOME PHASES OF THE RELATION OF TEMPERATURE TO THE DEVELOPMENT OF INSECTS. W. Va. Agr. Expt. Sta. Bul. 208, 62 pp., illus.
(14) POWSNER, L.
1935. THE EFFECTS OF TEMPERATURE ON THE DURATIONS OF THE DEVELOPMENTAL STAGES OF DROSOPHILA MELANOGASTER. Physiol. Zool. 8: 474–520, illus.
(15) TITSCHACK, E.
1925. UNTERSUCHUNGEN ÜBER DEN TEMPERATUREINFLUSS AUF DIE KLEIDERMOTTE (TINEOLA BISELLIELLA HUM.). Ztschr. f. Wiss. Zool. 124: [213]–251, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., AUGUST 15, 1944 No. 4

RELATION OF CULTIVATION TO DEPLETION OF ROOT RESERVES IN EUROPEAN BINDWEED AT DIFFERENT SOIL HORIZONS[1]

By A. L. BAKKE, *research professor*, W. G. GAESSLER, *research associate professor, Iowa Agricultural Experiment Station;* L. M. PULTZ, formerly *plant physiologist,* and S. C. SALMON, *principal agronomist, Division of Cereal Crops and Diseases' Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

It is well known that the roots of the European bindweed (*Convolvulus arvensis L.*) penetrate to considerable depths in the soil and that control by cultivation is effected by depletion of the root reserves. A number of investigators, including Bakke, Gaessler, and Loomis (*2*),[2] Frazier, as cited by Timmons (*9*), Bioletti (*5*), and Kiesselbach et al. (*7*), have reported roots at depths of 15 to 25 feet. Timmons (*9*) found very few roots below 6 feet in the dry upland soil at Hays, Kans.

Most root reserve studies, including those reported by Bakke et al. (*2*), Barr (*3*, *4*), and others, have been limited for the most part to the upper foot or 2 feet of soil. Obviously, such studies with European bindweed take into account only a part of the entire root system. It is important to know whether the roots in the upper soil layers are representative of the whole since excavation of all roots of such a deep-rooted plant is virtually impossible and removal of even a major portion is an arduous task. The primary objective of the work reported in this paper was to obtain this information. Second objective was to secure such data as might at the same time be obtained on the concentration and rate of depletion of reserves, and the death of the bindweed roots at various soil levels.

MATERIAL AND METHODS

COLLECTING THE ROOTS

The problem was approached by excavating the roots at various intervals on plots that were being cultivated for bindweed eradication at the Cherokee State Hospital farm, Cherokee, Iowa. The roots were kept separately for each soil level and analyzed for dry-matter content, reducing sugars, starch equivalent, and nitrogen.

The field on which the experiments were carried out is reported by employees of the State hospital to have been infested with bindweed for more than 20 years. The area of bindweed infestation is extremely variable, however, suggesting that most of the bindweed came by gradual spread from a few original patches rather than by seed, as in seed grain, for example. At the inception of the experimental work in 1936, the area was carefully surveyed and the infestation, based on visual inspection of the above-ground growth, recorded. Only the most heavily and uniformly infested areas were used in these studies. Three areas designated as plots 42, 72, and 142, each 4 by 2 rods, were chosen for the work and from these excavations were made and bindweed roots removed for analysis.

---

[1] Received for publication February 23, 1943. Contribution from the Iowa Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture and Four State Board of Control. Journal Paper No. J-1093 of the Iowa Agricultural Experiment Station, Project 484.

[2] Italic numbers in parentheses refer to Literature Cited, p. 147.

These plots were chosen because they were uniformly infested and appeared to be suitable for the purpose and because they were available at the time the excavations could be made. They were not intended as replicates of each other nor were they chosen to compare the root reserves under different treatments, though it is possible that some useful information might be obtained by comparing the results from the three plots.

The area had been used for general farming before the plots were laid out in 1936. Plot 42 was fallowed in 1937; i. e., for 1 year before the first excavations were made. The others prior to the first sampling date were handled no differently than might be expected on a well-managed farm, though somewhat better than on many bindweed-infested farms. The first excavations were made on plots 42 and 72 in May 1938 and on plot 142 in May 1939. After the first excavations the remainder of each plot was cultivated at frequent intervals (2 weeks or less) for the duration of the study. Sampling of plots 42 and 142 was continued for 2 years and sampling of plot 72 for 3 years. At the end of these periods practically all the bindweeds were dead.

Three or more excavations were made each year until the bindweeds on the unexcavated portions of the plots were dead or until it was no longer possible to get sufficient roots for analysis. The first sampling in each area was made at about the time the leaf-bearing shoots first appeared above the ground, the second in late June or early July at the approximate date of maximum above-ground growth, and the last in October or early November when growth had ceased. In 1939 and 1940, excavations were made in August also.

All excavations were 5x5 feet and as deep as roots were present in sufficient quantities to provide material for a chemical analysis from each soil level, i. e., about 4 gm. of dry roots per 25 cubic feet of soil. This depth was 8 feet for plots 42 and 72, and 6 feet for plot 142 for the first sample. At some of the later dates the quantity of roots at the lower depths was not sufficient for analysis.

The locations of the excavations made in each plot were selected at random, except that each excavation after the first was made at a sufficient distance from the earlier ones to avoid any effects on the bindweed that might have resulted from tramping and the disposition of material from the hole. The soil was removed in 1-foot layers, and during the process of removal the bindweed roots were separated by hand and placed in a 2-gallon milk can which was kept covered with a wet towel to prevent evaporation. Only living roots or living portions of roots were taken. These roots were then carefully washed to remove adhering soil particles, dried with toweling or cheesecloth until the surface was dry, weighed, subjected to live steam for 10 minutes to kill them, and immediately placed in a Hixon and Bakke field dehydrator (*6*) where they were left until completely dry. The dry material was later sent to the plant chemistry subsection, Iowa State College, for analysis.

CHEMICAL ANALYSIS

The samples as received were nearly, if not completely, air-dry. They were ground in a Wiley mill through a 40-mesh screen. The moisture content of the air-dried sample was determined by drying in a vacuum oven held at 70° C. and 25 to 28 inches of mercury for 14 hours. If the samples were less than 10 gm. in weight, a micro mill was used. A normal, complete analysis required 6 gm. of dry material. All determinations were made in duplicate. A 1-gm. sample sufficed for both the moisture and the nitrogen determinations, while 2 gm. were sufficient for both the sugar and the starch.

The sample from the moisture determination was used directly for the Kjeldahl (*1*) determination of nitrogen. The sample from the weighing bottle was conveniently transferred directly to a filter paper and inserted into a Kjeldahl flask. One mercury pill (consisting of 0.7 gm. mercuric oxide) was placed in each flask, as a catalyst. The remaining steps in the procedure are given in the Official Methods of Analysis (*1*).

The sugars were extracted from a 2-gm. aliquot sample of the ground material, being transferred to a Bailey-Walker thimble fitted with a 9-cm. filter paper; 30 cc. of 80-percent alcohol was added and the sample was extracted on a Baily-Walker extraction apparatus for 12 hours, cleared with neutral lead acetate, and deleaded with solid sodium oxalate, freed from alcohol, hydrolyzed with HCl at 25° C., and the total reducing sugars determined by the Shaffer and Hartmann method (*8*).

The residue from the sugar extraction was transferred to a 400-cc. beaker and gelatinized by autoclaving at 120° C. for 15 minutes at 15 pounds pressure. Malt diastase was used to digest the starch, and the filtrate was hydrolyzed with HCl without clearing. The reducing value of neutralized extract was also determined as before by the Shaffer-Hartmann method. The resulting values are here referred to as starch equivalent.

The nitrogen, reducing sugars, and starch-equivalent determinations are reported herein as percentage of dry matter. The reducing sugars and starch-equivalent determinations were added together and are here referred to as total "available" carbohydrates, though it is recognized that all the materials so designated may not be available to the plant.

EXPERIMENTAL RESULTS

The quantity of bindweed roots per cubic foot of soil, the percentage of dry matter, the total available carbohydrates expressed as percentage of the dry weight of roots, and per cubic foot of soil for plot 42 are presented in table 1. Similar data for plots 72 and 142 are given in tables 2 and 3. The total available carbohydrates and the quantity of roots are shown graphically in figures 1 and 2. The nitrogen content expressed as percentage of the dry matter, and the proportion of reducing sugars and starch equivalent varied considerably according to the depth of the roots and the time of sampling. Since they appeared to have no important bearing on the objectives of this study, the data are omitted in the interests of brevity. The nitrogen content of the roots of plot 142 at all depths and all sampling dates was from about 1.5 to 4 times greater than on plots 42 and 72. A reviewer of this paper suggests that the rapidity with which the bindweed on plot 142 were killed by cultivation may have been due to this fact. This cannot be denied although it would appear that the smaller quantity of roots and of available carbohydrates at the outset is an equally probable explanation.

The relative concentration of total available carbohydrates in different soil levels, and the rate of depletion of these carbohydrates and of roots, as may most readily be seen in figures 1 and 2, are substantially alike for all three plots. Hence the results for all of them can conveniently be discussed at one time.

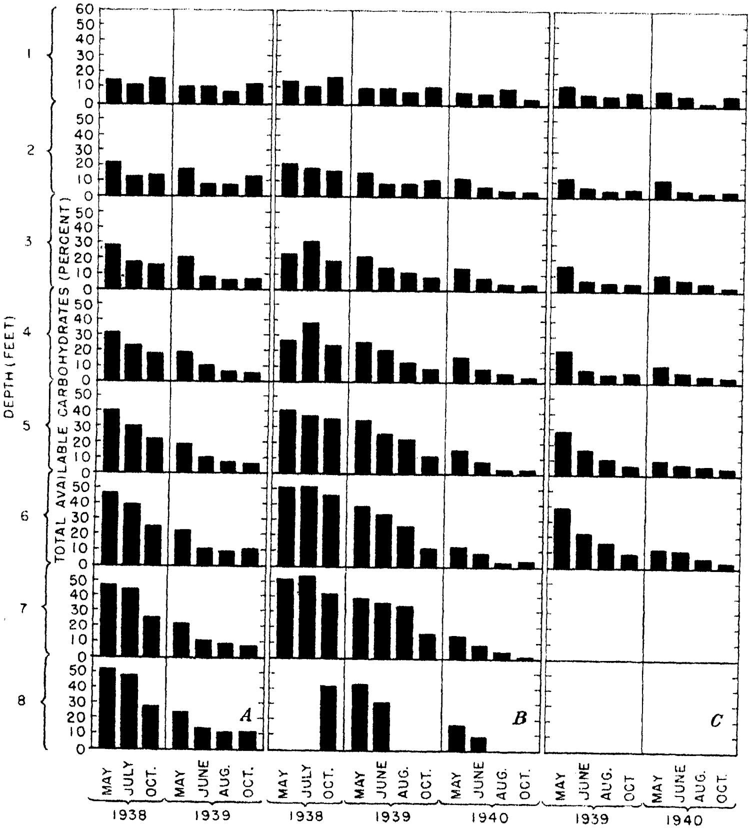


FIGURE 1.—Total available carbohydrates expressed as percentage of the dry weight in the roots of European bindweed at various depths in the soil: *A*, Plot 42; *B*, plot 72; and *C*, plot 142, at Cherokee, Iowa, 1938, 1939, and 1940.

Perhaps the most significant facts shown by these data are the high concentration of available carbohydrates in the lower soil levels (fig. 1) and the concentration of roots in the upper levels (fig. 2). The former increases and the latter decreases with depth. The rate of change in root quantity is greater than that for percentage of available carbohydrates and consequently the quantity of the latter (tables 1 to 3) expressed as grams per cubic foot of soil is much greater in the surface or near-surface levels.

On the first sampling date the surface foot of the freshly excavated roots contained 24.6 percent of all the roots in plot 42, 27.0 percent in plot 72, and 24.7 percent in plot 142. Corresponding figures for the upper 2 feet are 47.2, 49.8, and 50.3 percent. The values for

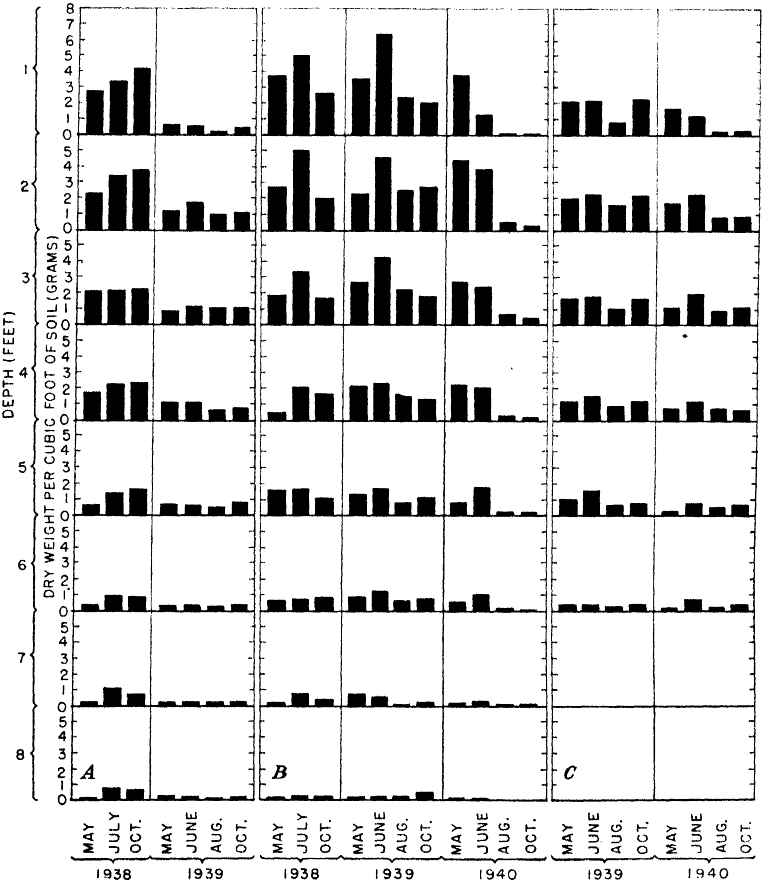


FIGURE 2.—Dry weight of roots of European bindweed in grams per cubic foot of soil at various depths in the soil: *A*, Plot 42; *B*, plot 72; and *C*, plot 142, at Cherokee, Iowa, 1938, 1939, and 1940.

available carbohydrates in the upper foot of the three plots were 18.4, 14.1, and 21.6 percent and for the upper 2 feet 32.6, 34.2, and 46.2 percent. It would appear, therefore, that in these plots sampling to a depth of 2 feet would have included about 50 percent of the roots and one-third or more of the available carbohydrates. It is probable that had the first samples been taken at a different time of the year or on plots so handled that the bindweed had had a better opportunity to grow, there would have been a larger proportion of roots and of the available carbohydrates in the upper foot or 2 feet of soil.

There was a constant decrease in quantity of roots and in percentage and quantity of carbohydrates with time, accompanied as in this case by cultivation. The first effect of the cultivation was to reduce the concentration of carbohydrates. This decrease continued in plot 42 until a low point was reached several weeks or months before the bindweed plants were killed. There were indications of a similar relation in plots 72 and 142, but the evidence is less conclusive. For a while there appeared to be no decrease in root weight other than could be ac-

TABLE 1.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 42*

Depth (feet)	1938			1939			
	May	July	Oct.	May	June	Aug.	Oct.
QUANTITY OF ROOTS (GRAMS DRY WEIGHT PER CUBIC FOOT OF SOIL							
1	2.77	3.46	4.26	0.80	0.62	0.30	0.55
2	2.38	3.48	3.88	1.31	1.82	1.02	1.15
3	2.08	2.20	2.34	.97	1.19	1.04	1.03
4	1.75	2.32	2.38	1.09	1.10	.64	.81
5	.63	1.45	1.64	.71	.58	.47	.92
6	.37	.99	.96	.37	.39	.28	.42
7	.27	1.02	.74	.27	.28	.24	.29
8	.15	.80	.76	.29	.26	.14	.24
Total	10.40	15.72	16.96	5.81	6.24	4.13	5.41
DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)							
1	12.0	13.7	20.6	17.3	12.4	13.4	17.6
2	17.5	20.3	21.7	20.3	20.4	18.1	19.6
3	24.0	18.3	25.4	20.4	18.5	17.5	20.6
4	25.7	17.7	25.3	17.9	16.2	15.8	19.1
5	23.5	17.3	16.8	18.6	14.8	15.1	17.1
6	21.8	17.0	14.7	16.7	13.8	12.8	15.6
7	24.7	18.5	13.4	15.3	12.5	12.8	14.2
8	21.5	22.5	13.5	14.7	13.3	12.3	12.0
TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)							
1	15.56	13.81	17.00	10.17	10.21	8.45	13.89
2	21.40	12.41	14.14	17.31	7.28	7.26	13.77
3	29.00	17.35	15.31	21.23	8.95	6.50	6.93
4	30.13	22.95	18.05	18.40	10.07	6.91	6.86
5	41.83	31.58	22.37	19.80	10.59	7.52	6.87
6	47.05	39.66	25.97	23.78	10.29	9.98	10.14
7	47.54	44.44	25.09	22.13	10.47	8.91	7.88
8	51.56	48.80	27.24	24.56	13.41	10.26	10.42
TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)							
1	0.431	0.478	0.724	0.081	0.063	0.025	0.076
2	.509	.432	.549	.227	.132	.074	.213
3	.603	.382	.358	.206	.107	.068	.084
4	.527	.532	.430	.201	.110	.034	.056
5	.263	.458	.167	.141	.061	.035	.063
6	.174	.393	.249	.088	.041	.027	.033
7	.127	.453	.186	.060	.029	.021	.023
8	.077	.390	.207	.071	.035	.014	.025
Total	2.711	3.518	2.870	1.075	.578	.298	.573

counted for by loss of carbohydrates. For example, on plot 42 the quantity of roots in the excavation made in October 1938 was actually greater than in the one made in May, a result, no doubt, of variability in bindweed infestation. In plots 72 and 142 there were no appreciable differences in the quantity of roots in May and in October of the first year of sampling. On the other hand, there was a distinct decrease in percentage of available carbohydrates on all plots. After the first year of cultivation both roots and percentage of available carbohydrates decreased.

TABLE 2.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 72*

Depth (feet)	1938			1939				1940			
	May	July	Oct.	May	June	Aug.	Oct.	May	June	Aug.	Oct.
QUANTITY OF ROOTS (GRAMS PER CUBIC FOOT OF SOIL											
1	3.82	5.08	2.57	3.51	6.40	2.46	2.10	3.94	1.37	0.03	0.03
2	2.81	5.10	2.07	2.30	4.58	2.50	2.74	4.40	3.80	.52	.41
3	1.98	3.47	1.67	2.87	4.30	2.30	1.90	2.79	2.48	.64	.45
4	1.00	2.19	1.71	2.22	2.40	1.61	1.40	2.27	2.05	.44	.26
5	1.68	1.71	1.18	1.42	1.78	.90	1.21	.90	1.80	.29	.24
6	.65	.68	.80	.88	1.24	.59	.84	.50	1.00	.21	.11
7	.21	.78	.42	.78	.63	.19	.25	.33	.38	.09	.09
8	.18	.35	.35	.25	.28	.28	.55	.20	.19		
Total	12.33	19.36	10.77	14.23	21.61	10.83	10.99	15.33	13.07	2.22	1.59
DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)											
1	20.7	21.4	20.3	17.9	17.9	19.6	18.2	24.8	18.0	31.88	16.73
2	25.4	25.6	22.4	26.3	24.2	21.9	22.9	25.1	22.7	23.72	18.65
3	26.1	27.2	21.8	23.3	21.5	19.4	20.4	23.2	20.1	22.75	22.68
4	28.0	25.7	20.3	23.2	19.7	17.5	18.4	20.9	16.9	18.19	18.61
5	28.3	28.0	19.5	22.4	19.6	15.9	16.8	17.0	16.0	16.17	17.18
6	29.3	30.3	20.1	24.7	20.7	15.7	14.8	16.8	15.2	13.10	16.93
7	23.9	28.4	22.1	21.9	21.7	16.9	14.1	16.1	15.4	13.90	17.78
8		29.0	22.2	23.8	20.5			14.7	14.9		
TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)											
1	15.96	12.81	19.28	10.77	10.85	8.99	11.96	8.37	7.59	10.18	4.11
2	20.96	18.74	16.83	15.69	9.82	9.85	11.01	11.72	6.78	4.21	3.54
3	23.62	30.92	19.04	20.45	14.77	10.73	8.03	14.12	7.74	4.25	3.46
4	27.06	38.49	24.12	25.08	20.06	13.24	8.23	15.55	7.80	6.01	3.33
5	41.90	37.91	35.46	35.01	25.14	22.05	11.64	15.22	8.59	2.80	2.81
6	50.78	51.35	46.71	38.10	33.79	26.84	12.43	13.05	9.48	2.87	3.94
7	51.95	53.50	42.72	39.30	36.16	34.60	16.35	14.96	9.26	4.20	2 36
8			42.45	42.57	31.16			15.26	8.21		
TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)											
1	0.610	0.507	0.495	0.378	0.694	0.121	0.251	0.330	0.104	0.003	0.001
2	.590	1.056	.348	.361	.439	.246	.302	.516	.258	.022	.015
3	.468	1.073	.318	.587	.635	.247	.153	.394	.192	.027	.016
4	.271	.822	.413	.552	.481	.213	.115	.353	.160	.026	.009
5	.704	.648	.418	.497	.447	.198	.141	.137	.165	.007	.007
6	.330	.349	.374	.335	.419	.158	.104	.065	.095	.006	.004
7	.109	.417	.179	.306	.228	.066	.041	.049	.035	.004	.002
8			.149	.106	.097			.031	.016		
Total	3.082	4.872	2.694	3.122	3.440	1.249	1.107	1.875	1.025	.095	.054

Data from plots 42 and 142 indicate a substantial loss between the last sampling dates in the fall and the first in the spring, which could have been due to growth in the spring before the first samples were taken, or to variation in root quantity and in reserves from one place to another in the field.

It frequently was observed in this study that the roots died from the top down. This is illustrated in figure 3 which shows a bindweed root that had been killed by cultivation to a depth of about 30 inches and another killed to a depth of 59 inches, each of which produced rhizomes that eventually reached the surface of the soil and produced leaves.

TABLE 3.—*Quantity of roots and dry matter, and total available carbohydrates in the roots of European bindweed in plot 142*

Depth (feet)	1939				1940			
	May	June	Aug.	Oct.	May	June	Aug.	Oct.
QUANTITY OF ROOTS (GRAMS PER CUBIC FOOT OF SOIL)								
1	2.16	2.16	1.41	2.30	1.74	1.34	0.26	0.28
2	2.04	2.30	1.56	2.31	1.78	2.30	.93	.94
3	1.70	1.87	1.06	1.72	1.08	1.98	.80	1.11
4	1.36	1.55	.98	1.34	.76	1.20	.70	.66
5	1.01	1.55	.68	.90	.44	.75	.48	.56
6	.46	.43	.31	.46	.22	.62	.27	.47
Total	8.73	9.86	6.00	9.03	6.02	8.19	3.44	4.02
DRY MATTER (PERCENTAGE OF THE WEIGHT OF FRESH ROOTS)								
1	19.9	17.3	15.5	17.9	18.2	16.9	20.04	11.76
2	24.9	20.5	19.7	22.2	21.6	22.1	21.81	20.44
3	24.7	23.0	18.2	20.7	21.0	20.5	19.91	20.12
4	26.4	20.5	17.3	18.1	22.9	18.2	17.73	19.68
5	17.3	20.6	18.5	16.4	20.3	17.2	16.84	16.36
6	20.5	22.3	18.7	18.7	18.2	15.3	15.69	14.93
TOTAL AVAILABLE CARBOHYDRATES (PERCENTAGE OF DRY WEIGHT OF ROOTS)								
1	0.285	0.157	0.098	0.212	0.162	0.074	0.010	0.019
2	.273	.125	.068	.109	.195	.090	.030	.035
3	.255	.108	.053	.074	.134	.101	.037	.033
4	.202	.133	.056	.077	.088	.072	.034	.025
5	.283	.262	.068	.052	.040	.047	.028	.025
6	.176	.096	.051	.043	.024	.068	.015	.015
Total	1.564	.881	.394	.567	.643	.452	.154	.152
TOTAL AVAILABLE CARBOHYDRATES (GRAMS PER CUBIC FOOT OF SOIL)								
1	13.20	7.28	6.92	9.22	9.33	5.50	3.39	6.86
2	13.39	5.43	4.39	4.74	10.94	4.36	3.25	3.74
3	15.01	5.75	4.95	4.32	12.42	5.12	4.61	2.98
4	21.49	8.60	5.76	5.79	11.59	6.00	4.95	3.81
5	27.97	16.90	10.04	5.79	9.19	6.25	5.77	4.55
6	38.20	22.28	16.59	9.35	10.99	10.92	5.44	3.25

Especially after a severe winter but not limited thereto, the roots may be dead to a depth of a foot or more. The depth to which it was necessary to dig in order to find live roots became greater as the cultivation period was extended. A plausible explanation for this would be the inability of the plant to translocate available carbohydrates upwards rapidly enough to keep pace with growth. If this is the correct explanation, the methods of the present study were not sufficiently refined to show it. Another possibility is that roots tend to die when the concentration of available carbohydrates falls below a certain minimum. In these studies the concentration in the upper part of the root was low to begin with as compared with that at greater depths and may have reached a level that was not sufficient to maintain life in the roots. Whether such was the case, however, cannot be determined from the data.

It appears to be significant, in relation to root-sampling studies, that the depletion in carbohydrates with cultivation seemed to take place as rapidly or even more rapidly at the lower levels than near the surface. The differences in rates of depletion are not great, however, and sampling to a depth of 2 feet only in these plots would have provided a substantially correct picture of the comparative reserves. The rate of depletion may be a function of the relative concentration at different levels, being more rapid at those soil horizons and in those cases where the concentration is greatest.

As mentioned above, the percentage of carbohydrates appears to reach a minimum level several weeks or months before all plants are dead and except for slight differences due to translocation from lower to upper levels seems to remain constant to the end within the limits of sampling error. This level was about 7 or 8 percent in plot 42, about 3 percent in plot 72, and 3 or 4 percent in plot 142. Probably much of this represents a systematic error in analysis or, more accurately, an error in designating as "available" the reducing sugar and "starch equivalent." Actually, as pointed out by Bakke, Gaessler, and Loomis (*2*), and recognized at the outset of this study, a small proportion of the material separated by the methods employed is not available to the plant. The error, however, is not regarded as important so far as the purpose of the present study is concerned. Why there should have been a larger quantity of this unused material in the roots of plot 42 than in the others is not known.

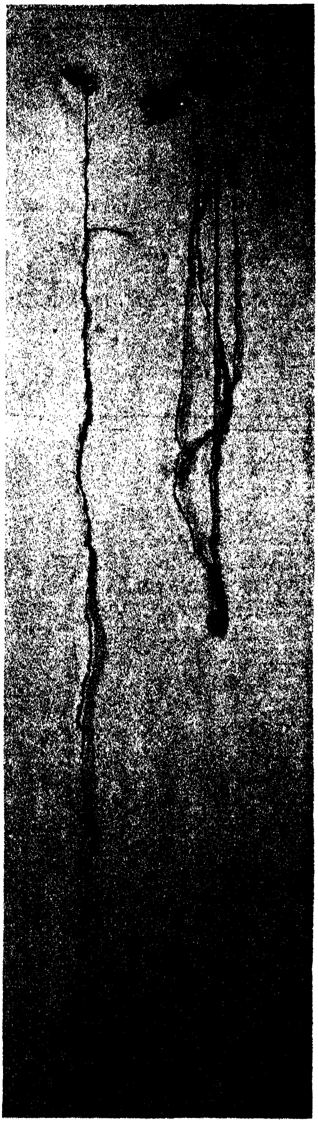


FIGURE 3.—Two European bindweed roots killed by cultivation to depths of 30 and 59 inches with rhizomes reaching the soil surface and above.

SUMMARY

The effect of cultivation on root quantity and on root reserves in the European bindweed (*Convolvulus arvensis* L.)at various depths in the soil was studied at the Cherokee State Hospital, Cherokee, Iowa, during a 3-year period. These studies were made on land that probably had been infested with bindweed for 20 years or more. The studies included three different plots. On two of them sufficient bindweed roots for chemical analysis (about 4 gm. of dry material from 25 cubic feet of soil) was found to a depth of 8 feet and on the third plot to a depth of 6 feet. The maximum penetration of roots was not determined. The study was begun on one of the plots after it had been intensively cultivated for one season, which probably reduced root reserves somewhat as compared with normally cultivated and cropped land. The cultivation and cropping on the others was more intensive than on most bindweed-infested fields though probably no more so than on well-managed farms.

The quantity of roots per unit volume of soil for each foot level was recorded, the dry-matter content of the roots was determined, and the dry matter was analyzed for reducing sugars, starch (starch equivalent), and nitrogen. The sugars and starch were added together for convenience and herein reported as total available carbohydrates, though it is recognized that a part of the material so designated may not be available to the plant. This is probably due in part at least to the method of analysis which is known to include some material other than sugar and starch.

Cultivation resulted in a gradual and continuous reduction in the concentration and depletion of the quantity of total available carbohydrates, a decrease in root quantity, and eventually in the death of the bindweed plants. It appeared that cultivation first affected the concentration of carbohydrates, the depletion in root weight being principally due to loss of carbohydrates. Later the roots began to die, resulting in an additional reduction of the weight of living roots.

The concentration of available carbohydrates markedly increased with depth, the quantity expressed as a percentage of dry matter being from two to four times as much in the lower as in the upper soil horizons. However, because of the much greater quantity of roots in the upper soil layers, the quantity of carbohydrates in these layers was also much greater than at the lower depths.

At the beginning of the sampling periods approximately 25 percent of the root quantity and 18 percent of the total available carbohydrates were found in the upper foot of soil and about 49 and 38 percent, respectively, in the upper 2 feet of soil.

General observations showed that the roots died first in the upper soil horizons, the least depth at which living roots could be found increasing with time. This suggests that the conversion of starch to sugar or the translocation of sugar from the lower levels was not sufficiently rapid to keep the roots alive. However, no consistent differences in the rate of depletion of starch or sugars at different depths such as would account for this could be demonstrated. In some cases the rate of depletion of carbohydrates was more rapid at the lower soil levels than near the surface, but in other cases the reverse seemed to be true.

An accurate picture of root reserves in European bindweed cannot be had without considering both the concentration of carbohydrates and root quantities in all soil horizons in which there are appreciable root quantities. Since, however, the greater proportion of roots and available carbohydrates are found in the upper layers of soil, it appears that a close approximation can be secured by sampling the upper 2 feet only.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1935. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus. Washington, D. C.

(2) BAKKE, A. L., GAESSLER, W. G., and LOOMIS, W. E.
1939. RELATION OF ROOT RESERVES TO CONTROL OF EUROPEAN BINDWEED CONVOLVULUS ARVENSIS L. Iowa Agr. Expt. Sta. Res. Bul. 254, pp. [113]-144, illus.

(3) BARR, C. G.
1936. PRELIMINARY STUDIES ON THE CARBOHYDRATES IN THE ROOTS OF BINDWEED. Amer. Soc. Agron. Jour. 28: 787-798, illus.

(4) ———
1940. ORGANIC RESERVES IN THE ROOTS OF BINDWEED. Jour. Agr. Res. 60: 391-413, illus.

(5) BIOLETTI, F. T.
1911. THE EXTERMINATION OF MORNING-GLORY. Calif. Agr. Expt. Sta. Cir. 69; 12 pp., illus.

(6) HIXON, R. M., and BAKKE, A. L.
1939. PORTABLE FIELD DRIER. (Note) Amer. Soc. Agron. Jour. 31: 268-270., illus.

(7) KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W.
1934. BINDWEEDS AND THEIR CONTROL. Nebr. Agr. Expt. Sta. Bul. 287, 47 pp., illus.

(8) SHAFFER, P. A., and HARTMANN, A. F.
1921. THE IODOMETRIC DETERMINATION OF COPPER AND ITS USE IN SUGAR ANALYSIS. Pts. I and II. Jour. Biol. Chem. 45: 349-390, illus.

(9) TIMMONS, F. L.
1941. RESULTS OF BINDWEED CONTROL EXPERIMENTS AT THE FORT HAYS BRANCH STATION, HAYS, KANSAS, 1935 TO 1940. Kans. Agr. Expt. Sta. Bul. 296, 50 pp., illus.

ADDITIONAL STRAINS OF THE SUGAR-BEET CURLY TOP VIRUS[1]

By N. J. GIDDINGS

Senior pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Four strains of the sugar-beet curly top virus were reported in 1938 [2] as having been clearly differentiated. At that time it was evident that there were other strains; the present paper reports the results of further work in the isolation and differentiation of these.

The term "selection," as applied to viruses in this paper, indicates that a virus was selected for further study, because of some peculiarity in host reaction or because of an unusual host or a new location, upon the assumption that such a selected virus might be found to be an undescribed strain of curly top. Therefore the term "selection" is considered more appropriate than either "isolation" or "culture."

When new selections of any virus are being used, there is always some uncertainty as to whether a single strain or a mixture of two or more strains may be present. Accordingly great care has been taken to avoid describing such strain combinations as new strains.

SOURCES OF VIRUS STRAINS

Six additional strains (Nos. 5, 6, 7, 8, 9, and 10) have been segregated from a large group of curly top virus selections. Strains 5, 6, and 10 were obtained in 1937 from plants of tree tobacco (*Nicotiana glauca* Graham) which had grown in natural breeding areas of the beet leafhopper (*Eutettix tenellus* (Bak.)) near Tracy, Calif. Strain 7 was obtained in 1937 from diseased spinach plants (*Spinacia oleracea* L.) growing in a commercial field west of Hemet, Calif. Strain 8 came under test in 1936 as a result of inoculations by J. M. Wallace[3] on Red Mexican bean (*Phaseolus vulgaris* L.) with a colony of viruliferous leafhoppers carrying a mixture of unknown virus strains. Strain 9 was obtained earlier from a similar colony by inoculations on a resistant beet (*Beta vulgaris* L.) but was first recognized as a distinct selection at the same time as strain 8 and as a result of the same group of inoculations.

---

[1] Received for publication March 3, 1943.

[2] GIDDINGS, N. J. STUDIES OF SELECTED STRAINS OF CURLY TOP VIRUS. Jour. Agr. Res. 56: 883-894, illus. 1938.

[3] Unpublished data from the records of J. M. Wallace, formerly of the Division of Sugar Plant Investigations.

Journal of Agricultural Research, Washington, D. C.

Vol. 69, No. 4
Aug. 15, 1944
Key No. G-1306

DIFFERENTIAL HOSTS

Plants tested for possible value as differential hosts include 47 species and varieties, besides a number of varieties of cultivated sugar beet (*Beta vulgaris*). These plants may be divided into two groups, one containing those that became infected by one or more strains of the curly top virus and the other those in which none of the virus strains produced infection.

SPECIES OR VARIETIES IN WHICH INFECTION WAS OBTAINED

CHENOPODIACEAE:
- *Beta macrocarpa* Guss.
- *Beta patellaris* Moq.
- *Beta trigyna* Waldst. and Kit.
- *Beta vulgaris*
- *Chenopodium murale* L. (nettleleaf goosefoot)
- *Spinacia oleracea* (spinach, var. Improved Prickly Winter)

COMPOSITAE:
- *Lactuca sativa* L. (lettuce, var. Los Angeles Head)

CRUCIFERAE:
- *Erysimum repandum* L.
- *Lepidium lasiocarpum* Nutt.
- *Lepidium nitidum* Nutt. (common peppergrass)
- *Malcolmia maritima* (L.) R. Br. (Virginian stock)

GERANIACEAE:
- *Erodium cicutarium* (L.) L'Hér. (redstem filaree)

HYDROPHYLLACEAE:
- *Nemophila maculata* Lindl. (spotted nemophila)

LEGUMINOSAE:
- *Lathyrus odoratus* L. (sweet pea)
- *Medicago hispida* Gaertn. (bur-clover)
- *Melilotus indica* All. (yellow sweetclover)
- *Phaseolus vulgaris* (bean, var. Bountiful)
- *Phaseolus vulgaris* (bean, var. Great Northern)
- *Phaseolus vulgaris* (bean, var. Red Mexican)
- *Trifolium hybridum* L. (alsike clover)
- *Trifolium incarnatum* L. (crimson clover)
- *Trifolium repens* L. (white clover)

PAPAVERACEAE:
- *Eschscholtzia californica* Cham. (California-poppy)

PLANTAGINACEAE:
- *Plantago erecta* Morris.
- *Plantago major* L. (common plantain)

PRIMULACEAE:
- *Samolus parviflorus* Raf. (water pimpernel)

SOLANACEAE:
- *Datura stramonium* L. (jimsonweed)
- *Lycopersicon esculentum* Mill. (tomato, var. Improved Stone)
- *Lycopersicon esculentum* (tomato, var. Earliana)
- *Lycopersicon esculentum* (tomato, var. John Baer)
- *Nicotiana glauca* (tree tobacco)
- *Nicotiana glutinosa* L.
- *Nicotiana tabacum* L. (tobacco, var. Turkish)
- *Solanum tuberosum* L. (potato, seedlings)

VIOLACEAE:
- *Viola tricolor* L. (pansy)

Some hosts in which infection was obtained were susceptible to all curly top strains tested on them, whereas others were infected by very few. The plants thus far found most useful in differentiating curly top virus strains are the susceptible sugar-beet selection S. L.[4] 842, the highly resistant sugar-beet selection 68,[5] Turkish tobacco, the highly resistant Red Mexican bean, and the very susceptible Bountiful bean.

SPECIES OR VARIETIES IN WHICH INFECTION WAS NOT OBTAINED

CRUCIFERAE:
- *Brassica adpressa* (Moench) Boiss.
- *Malcolmia africana* (L.) R. Br.

LEGUMINOSAE:
- *Medicago sativa* L. (alfalfa)
- *Trifolium pratense* L. (clover, var. Mammoth)
- *Trifolium pratense* (clover, var. Red)
- *Trifolium repens* L. (white clover, var. Ladino)

MALVACEAE:
- *Gossypium hirsutum* L. (cotton)
- *Hibiscus esculentus* L. (okra, var. Early Greenpod)

MYRTACEAE:
- *Feijoa sellowiana* Berg (pineapple guava)

PUNICACEAE:
- *Punica granatum* L. (pomegranate, seedlings)

ROSACEAE:
- *Malus* sp. (apple, var. Winesap, seedlings)
- *Rosa chinensis* Jacq. (rose, var. Ragged Robin, seedlings)

METHODS

The general methods used in studies to differentiate these strains were the same as those described in the report by the author on the first four strains,[6] except for important changes in the method of inoculating beans. In 1937, J. M. Wallace [7] inoculated Red Mexican bean with curly top virus and secured good evidence of infection and injury. The author had reported [6] that no case of infection was obtained by inoculation of Red Mexican bean with any curly top strains listed at that time; so notes and methods were compared. All the author's inoculations had been made before the second leaf opened out, and the viruliferous leafhoppers were caged [8] on the first or second (unfolding) leaf. Wallace had made inoculations before any leaf opened out by placing a large celluloid cage over the entire young plant. The leafhoppers were thus able to feed upon the cotyledons very soon after they appeared above ground. Wallace discovered that certain strains of the curly top virus would produce infection of Red Mexican bean if the leafhoppers fed upon the cotyledons, but that the same strains would not produce infection if the leafhoppers were allowed to feed only on young leaves of the plant. After learning this the writer made all bean inoculations so that the leafhoppers could feed on the young cotyledons. Wallace's inoculations on Red Mexican bean showed that the two curly top strains now designated as 8 and 9 produced distinctly more severe symptoms on that host than any of the other virus strains available at that time.

---

[4] Designation of seed lot at the Salt Lake City Station, Salt Lake City, Utah, of the Division of Sugar Plant Investigations.

[5] Formerly designated as strain 1167.

[6] See footnote 2.

[7] See footnote 3.

[8] GIDDINGS, N. J. A SMALL CAGE FOR INSECT VECTORS USED IN PLANT INOCULATIONS. Phytopathology 29: 649-650, illus. 1939.

All evidence here reported in relation to strain differentiation is the cumulative result of repeated inoculations with leafhoppers that had fed for some time on a plant infected with a given virus strain and is taken from records that include percentage of plants infected, relative severity of symptoms, and plant mortality. The severity rating is based on a scale of five grades, in which 1 indicates slight symptoms and 5 indicates very severe symptoms. Earlier published descriptions and illustrations of these grades of severity by the author[9] require some revision in view of newly discovered symptoms. The earlier and the revised description for each grade are given in table 1. The two types of grade 5 symptoms are shown in figure 1.

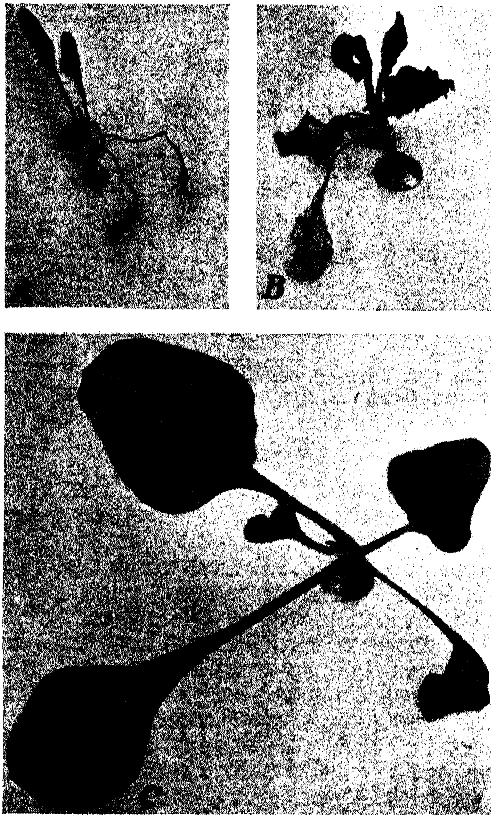


FIGURE 1.—Susceptible sugar-beet plants 40 days after infection by curly top virus, strain 6 (*A*) (*+++ in table 2) and strain 3 (*B*) (+++ in table 2); *C*, healthy check plant.

---

[9] See footnote 2.

TABLE 1.—*Comparison of original and revised descriptions of curly top severity grades*

Description	Curly top severity grade No. —				
	1	2	3	4	5
Original	Either slight vein clearing or occasional papillate growths on under side of foliage, or both.	Either slight curling of leaves with pronounced vein clearing or numerous papillae, or both.	Pronounced curling and some dwarfing.	Pronounced curling and increased dwarfing.	Extreme curling and dwarfing.
Revised	do	Generally as above. Newly discovered symptoms may cause confusion because of relatively little distortion even in plants in severity grades 3, 4, or 5, which are usually a darker green.	As above, or dwarfing without pronounced curling, but usually a darker green.	As above, or increased dwarfing without pronounced curling, but usually a darker green, with a rather high mortality rate.	As above, or extreme dwarfing without much curling, but usually a darker green, with a very high mortality rate.

EXPERIMENTAL RESULTS

EVIDENCE INDICATING THAT THE SELECTIONS ARE NEW STRAINS

Strains 5 and 6, when inoculated into young susceptible beets, induce characteristic symptoms quite different from those considered usual for curly top on that host. Infected small plants usually show little or no leaf distortion (fig. 1) and frequently have a deeper green color than is found in other diseased or healthy plants. There is extreme dwarfing of infected plants and a very high percentage of mortality among them. Plants infected in the three-leaf stage or later are not so likely to show the typical symptoms just described. Strain 5 has been most easily differentiated from strain 6 by the greater severity of symptoms it induces on Turkish tobacco (fig. 2). Strain 5 appears to be more virulent than strain 6 on *Nicotiana glutinosa*, whereas strain 6 seems more virulent than strain 5 when inoculated into Red Mexican bean, University of Idaho No. 15 selection of Great Northern bean, and the highly resistant sugar-beet variety 68.

Strain 7 is distinctly the least virulent of any thus far selected, and it is the only strain that has never given at least an occasional symptom on the resistant beet variety 68. It does not infect Turkish tobacco and only rarely infects *Nicotiana glutinosa*, producing very mild symptoms that are scarcely discernible. The curly top susceptible bean variety Bountiful, inoculated through the cotyledons, was rather quickly killed by most of the virus strains thus far tested; but plants of this variety infected by strain 7 were rarely killed and most of them made further growth (fig. 3) after pronounced curly top symptoms had been in evidence for some time. Infected spinach plants are usually severely stunted or killed by infection with any of the curly top strains except strain 7. It induces appreciable injury but much less than that caused by any of the other strains.

Strains 8 and 9 are very similar in virulence on most host plants tested, but strain 9 infects a higher percentage of plants of the resistant beet 68 and produces distinctly more severe symptoms than strain 8 upon the Red Mexican bean (fig. 4).

Strain 10, when inoculated into the young susceptible sugar-beet plants, produces symptoms quite similar to those induced by strains 5 and 6; but the severity is much less in the case of strain 10, and very few of the infected plants die. Its general host range is the same as that of strains 5 and 6, but the symptoms are much less severe on each of the hosts thus far tested, with the possible exception of tomato.

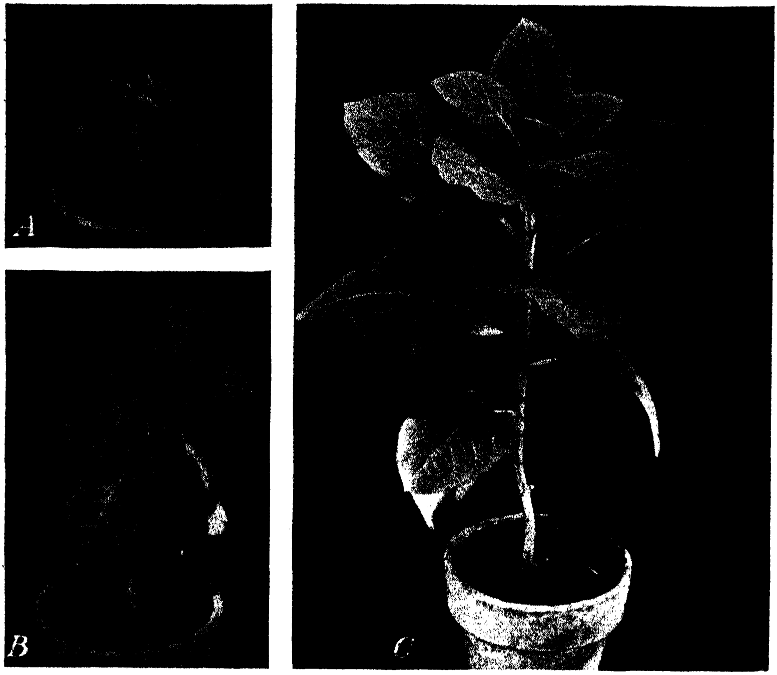


FIGURE 2.—Turkish tobacco plants 39 days after infection by curly top virus, strain 5 (*A*) and strain 6 (*B*); *C*, healthy check plant of same age.

Table 2 indicates the differential host reactions by which these strains and the previously described strains 1, 2, 3, and 4 may be distinguished. For presenting evidence on infection, the symbol + indicates that a high percentage of plants were infected, — indicates that a small percentage of plants were infected, and 0 that there were no plants showing symptoms. The severity of symptoms is classed as extremely slight (— —), slight (—), moderately severe (+), severe (++), and very severe (+++). Although these symbols are less precise than the means of a series of numerical grades, they are convenient classifications into which the actual readings are easily translated and constitute a much more usable method of expressing the differential reactions obtained with these virus strains.

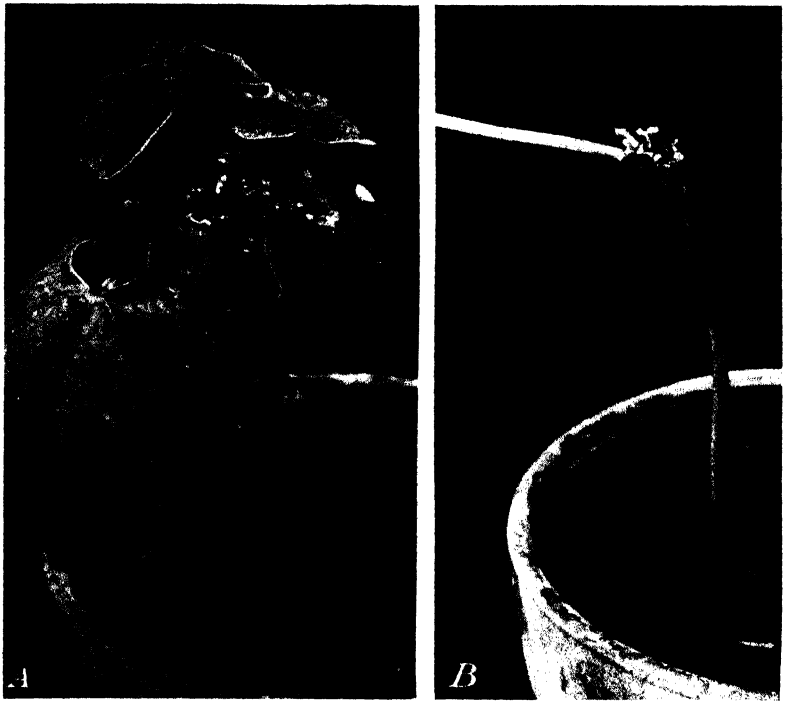


Figure 3.—Plants of the highly susceptible bean variety Bountiful 56 days after inoculation in cotyledon with curly top virus: *A*, Plant infected by virus strain 7 has survived and developed some new growth although it still shows very severe symptoms; *B*, plant infected by virus strain 2, which is less virulent than any of the other strains tested on Bountiful bean except strain 7.

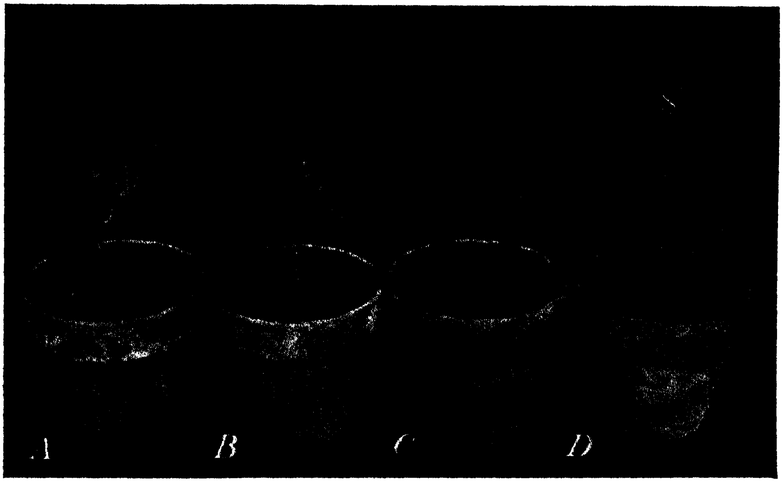


Figure 4.—Plants of the highly resistant bean variety Red Mexican 20 days after inoculation in cotyledon with different strains of curly top virus: *A*, Strain 3; *B*, strain 8; *C*, strain 9; *D*, uninoculated check.

The data upon which table 2 is based have been evaluated statistically, and all differences are highly significant.

TABLE 2.—*Key for differentiating 10 strains of curly top virus by means of a susceptible variety (S. L. 842) and a highly resistant variety (68) of sugar beets, Turkish tobacco, and Red Mexican bean*

Strain	Susceptible sugar beet		Resistant sugar beet		Turkish tobacco		Red Mexican bean	
	Infection[1]	Severity of symptoms[2]	Infection[1]	Severity of symptoms[2]	Infection[1]	Severity of symptoms[2]	Infection[1]	Severity of symptoms[2]
1	+	++	+	+	+	++	−	−
2	+	−	+	−	0	0	−	− −
3	+	+++	−	−	+	++	+	−
4	+	−	−	−	0	0	−	− −
5	+	*+++	−	−	+	+++	−	− −
6	+	*+++	−	−	+	++	+	−
7	+	− −	0	0	0	0	0	0
8	+	++	−	−	+	++	+	+
9	+	+++	+	−	+	++	+	++
10	+	*+	−	−	+	−	−	− −

[1] 0 = no plants infected; − = low percentage of plants infected; + = high percentage of plants infected.

[2] − − = extremely mild symptoms; − = mild or slight symptoms; + = moderately severe symptoms; ++ = severe symptoms; +++ = extremely severe symptoms; * = not much distortion of infected plants by strains 5, 6, or 10 but high mortality in case of strains 5 and 6.

STABILITY OF THE VIRUS STRAINS

Each of the curly top virus strains mentioned has been carried for several years, has been tested occasionally, and has shown a high degree of stability. Despite the stability of all these strains, any one of them may induce symptoms that show distinct differences in severity. For example, 20 susceptible plants infected by strain 7 and held under the same environmental conditions may quite commonly show 3 or 4 plants with symptoms graded as 2.0 on the scale of severity, a few graded as 1.5, and the remainder as 1.0. Such variation may be due to inherent differences between the individual plants or to environmental factors. In the case of strains 2, 4, and 7, there have been repeated selections of plants that showed a severity grade higher than the average for the strain under consideration, and in no case have any of these yielded a virus more virulent than the original selection of that strain. Similarly, there have been selections from the less severely diseased plants infected by strains 1 and 3, and in no case was there good evidence that a less virulent strain was obtained by this method.

A further effort was made to increase the virulence of strains 2, 4, and 7 by inoculation of very young susceptible plants and successive transfers from such plants just as soon as the first symptoms appeared. There was no evidence of increased virulence in any case.

DISCUSSION AND SUMMARY

The 10 virus strains reported in this paper are considered to be a part of a much larger group having the same vector, the same general host range, and similar symptoms. They may, therefore, be considered under one group designation as curly top virus, ***Ruga verrucosans*** Carsner and Bennett.[10]

---

[11] CARSNER, E., and BENNETT, C. W. NAME AND CLASSIFICATION OF THE CURLY-TOP VIRUS. Science 98: 385–386. 1943.

Each of the strains appears to be an entity which may be recovered from mixtures of strains and seems very stable. Because of the characteristics that they have in common, and as a matter of convenience, it seems best to designate these selections by strain number rather than as varieties of the species *Ruga verrucosans*.

A highly susceptible beet or other plant is essential for recognizing the presence of the virus. Any of the curly top resistant beets now available would readily serve to test for the presence of strain 1 or 2 but might not give any clear evidence concerning such highly virulent strains as 3, 5, or 6.

Host plants that effectively differentiate the various strains are necessary for progress in separating and classifying them, and more such plants are being sought. The 10 strains already differentiated present a picture of the plant reactions that have thus far been recorded.

The present paper describes a previously unidentified reaction, which consists of extreme dwarfing of young, susceptible sugar-beet plants without the pronounced distortion hitherto considered the chief symptom of severe injury. A high percentage of mortality among infected sugar-beet plants is usually associated with this newly described symptom.

The mere fact that a virus strain produces severe symptoms on one host is no definite indication as to the reaction that may occur with other hosts. Arrangement of the 10 described strains according to an ascending scale of amount of injury induced in the susceptible sugar beet would be approximately 7, 2, 4, 10, 1, 8, 3, 9, 5, 6. A similar arrangement according to injury in the resistant beet variety 68 would be 7, 10, 3, 8, 5, 6, 4, 2, 9, 1. Strain 7 holds the same relative position for each of these 2 beets, but strain 2 moves from second place on the susceptible beet to eighth place on the resistant beet. Strains 4 and 1 also show a much higher relative severity rating on the resistant beet than on the susceptible. On the other hand, strains 3, 5, and 6, which produced very severe symptoms on the susceptible beet, were relatively low in their rating on the resistant beet. Strains 2 and 4, which rate relatively high on the resistant beet, and strain 7, which is low on both beets, form a group that has never produced infection on Turkish tobacco. However, strain 10 produces mild symptoms, strain 6 rather severe symptoms, and strains 1, 3, 5, 8, and 9 produce very severe symptoms on that host. On the Red Mexican bean, strains 8 and 9 were the only ones that induced comparatively severe injury and strain 9 was clearly more virulent than strain 8.

Adequate knowledge of various curly top strains has an important bearing upon the disease-resistance breeding program for other host plants as well as for sugar beets.

BIOLOGY OF ALLOTROPA BURRELLI, A GREGARIOUS PARASITE OF PSEUDOCOCCUS COMSTOCKI[1]

By D. W. Clancy

Associate entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

In the fall of 1939 a shipment of a gregarious platygasterid, *Allotropa burrelli* Mues.,[2] was received at Moorestown, N. J., from the Yokohama, Japan, station of the Division of Foreign Parasite Introduction, Bureau of Entomology and Plant Quarantine. The primary purpose of the importation was to furnish breeding stock for propagating parasites of this species for release against the Comstock mealybug (*Pseudococcus comstocki* (Kuw.)), which has recently become a serious pest of apple in sections of Virginia, West Virginia, and Ohio.

During the course of the propagation work at Moorestown, from January 1940 to January 1941, the author had an excellent opportunity to make observations on the biology of this species, a member of a very interesting and little-known group. The genus *Allotropa* contains only three other described North American species, *A. convexifrons* Mues. from the Comstock mealybug,[2] *A. utilis* Mues. from *Phenacoccus aceris* (Sign.),[3] and *A. ashmeadi* Mues. from an undetermined mealybug.[3]

A special attempt was made to determine whether polyembryony occurs in this platygasterid. All drawings and observations were made from fresh material dissected in normal salt solution, without special stains or sectioning. Consequently, the precleavage development of the egg was not followed.

THE ADULT

The adult is shining black and approximately 0.8 mm. long. The sexes can be most readily distinguished by differences in the antennae; the male has long, hirsute, moniliform antennae, whereas the female has shorter, sparsely pubescent, and distinctly clavate antennae. The insects are fairly active at room temperatures, although they rarely attempt flight.

At 80° F. adults live on an average but 3 to 5 days. Even at lower temperatures they seldom live more than 10 days.

Males generally begin emerging first and remain on or near the mummies[4] to assist and mate with the females. The eggs are deposited at random in the host's body cavity, and oviposition begins immediately if hosts are present. After a brief examination, the

---

[1] Received for publication November 24, 1942.

[2] Muesebeck, C. F. W. TWO NEW SPECIES OF ALLOTROPA (PLATYGASTERIDAE, SERPHOIDEA) PARASITIC ON THE COMSTOCK MEALYBUG. Brooklyn Ent. Soc. Bul. 37, pp. 170-173, illus. 1942.

[3] Muesebeck, C. F. W. A NEW MEALYBUG PARASITE (HYMENOPTERA: SCELIONIDAE). Canad. Ent. 71: 158-160, illus. 1939.

[4] In this paper the term "mummy" refers to the inflated host derm containing parasite larvae or pupae within the typical hardened cells.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 4
Aug. 15, 1944
Key No. K-329

female turns about, thrusts her ovipositor backward into the mealybug, and then remains motionless for 5 to 10 seconds, often being dragged along by the host until oviposition is completed. Under favorable conditions the female parasitizes several hosts in quick succession, thereby depositing most of her eggs in a short time—an obvious necessity in view of her short life span.

Mealybug nymphs of all stages are attacked, although preference appears to be shown for those at least half grown. Small hosts are generally able to become nearly mature before being mummified, although the developmental period is somewhat lengthened.

THE EGG AND THE PARASITE BODY

The ovarian eggs are exceedingly small and numerous; they average 0.056 by 0.010 mm. in size (fig. 1, *A*). From 332 to 784 eggs per female, with an average of 565, have been disclosed by dissection. The ovarian eggs are nearly all mature when the female emerges, although the ovarioles may produce additional ova as deposition proceeds.

After oviposition at 75°-80° F., the deposited egg remains outwardly unchanged for approximately 24 hours. Within the next 12 hours, however, it commences to shorten and becomes more spherical in outline, until after 36 to 48 hours it is perfectly round and measures about 0.025 mm. in diameter (fig. 1, *B*). At this stage the central body is well defined, either round or oval, and distinctly granular in appearance. It is obviously identical with the "masse vitelloide" of the monembryonic platygasterid *Synopeas rhanis* Walk.,[5] although the embryonic nuclei are not yet apparent. It is surrounded by a circular clear area which is variable in extent (fig. 1, *B* and *C*), and outside of that is a ring of gray, semiopaque material which is probably the trophamnion. Eggs in this stage can be distinguished from the fat cells and other of the host contents only by their typical organization. Owing to the quantity of host tissue encountered, it was impossible to identify eggs prior to this stage after they had become spherical in outline.

Intermixed with these eggs are numerous others that have attained the morula stage (fig. 1, *D*), together with many in the premorula stage (fig. 1, *B*). All stages are present approximately 2 days after oviposition, owing to individual differences in rate of development. Since the egg begins to increase in size during the morula stage, it is henceforth known as the parasite body. It now averages 0.036 mm. in diameter, and generally contains five or six embryonic nuclei around the central body. It will be noted that the entire embryonic region is slightly off center. The paranucleus was not observed in the temporary unstained mounts used, although on several occasions faint partial outlines of the paranucleus were noted.

After about 2½ days the parasite body enters the early blastula stage (fig. 1, *E*) and measures approximately 0.045 mm. The paranucleus is now a distinct round to oval-crescentic body located in the wider portion of the trophamnion. The embryonic nuclei vary in number, and each consists of an inner and an outer layer; this double-bodied effect is also frequently noticeable among late morulae.

---

[5] MARCHAL, P. RECHERCHES SUR LA BIOLOGIE ET LE DÉVELOPPEMENT DES HYMÉNOPTÈRES PARASITES LES PLATYGASTERS. Arch. de Zool. Expt. et Gén. (IV) 4: 485-640, illus. 1906.

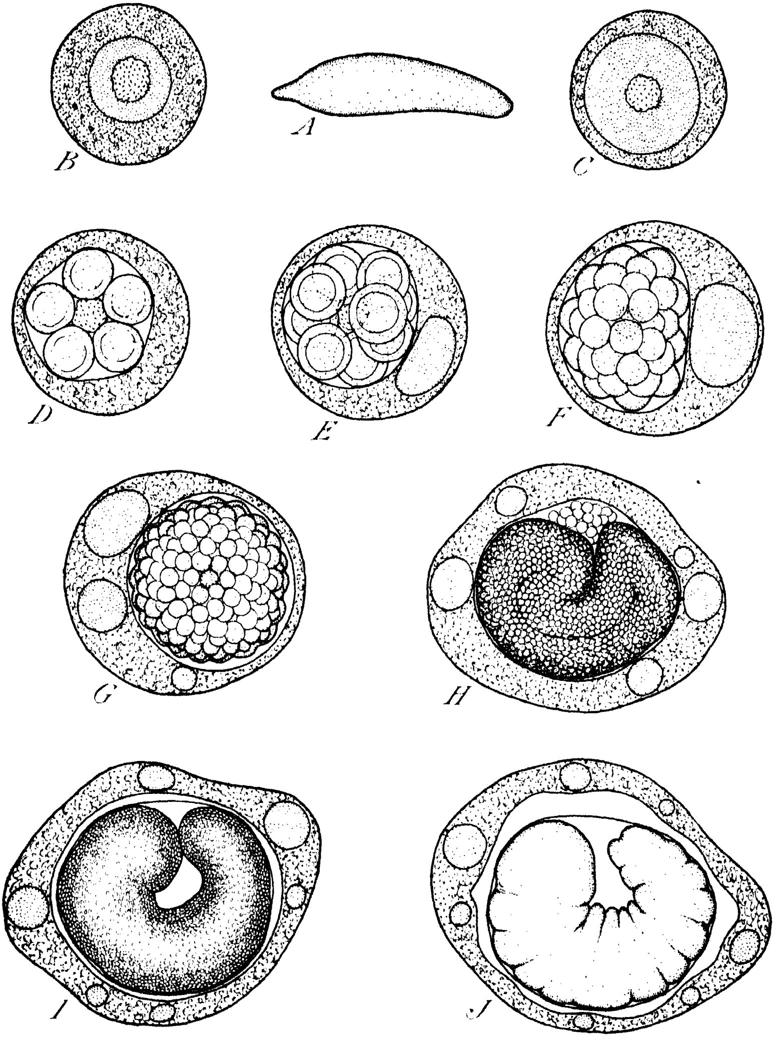


Figure 1.—Embryonic development of *Allotropa burrelli: A*, Ovarian egg; *B* and *C*, premorulae before appearance of embryonic nuclei; *D*, typical morula; *E*, early blastula, showing double-bodied embryonic nuclei and paranucleus; *F*, later blastula; *G*, late blastula with three paranuclear masses; *H*, gastrula, showing proliferated cells; *I*, later embryo undergoing organ and tissue formation; *J*, advanced embryo within delicate membrane.

About 3 days from oviposition the typical hollow sphere or blastula becomes apparent, generally with a single, large paranucleus in the periphery (fig. 1, *F*). The parasite body now averages 0.060 mm. in diameter, and is readily distinguished from the surrounding host bodies.

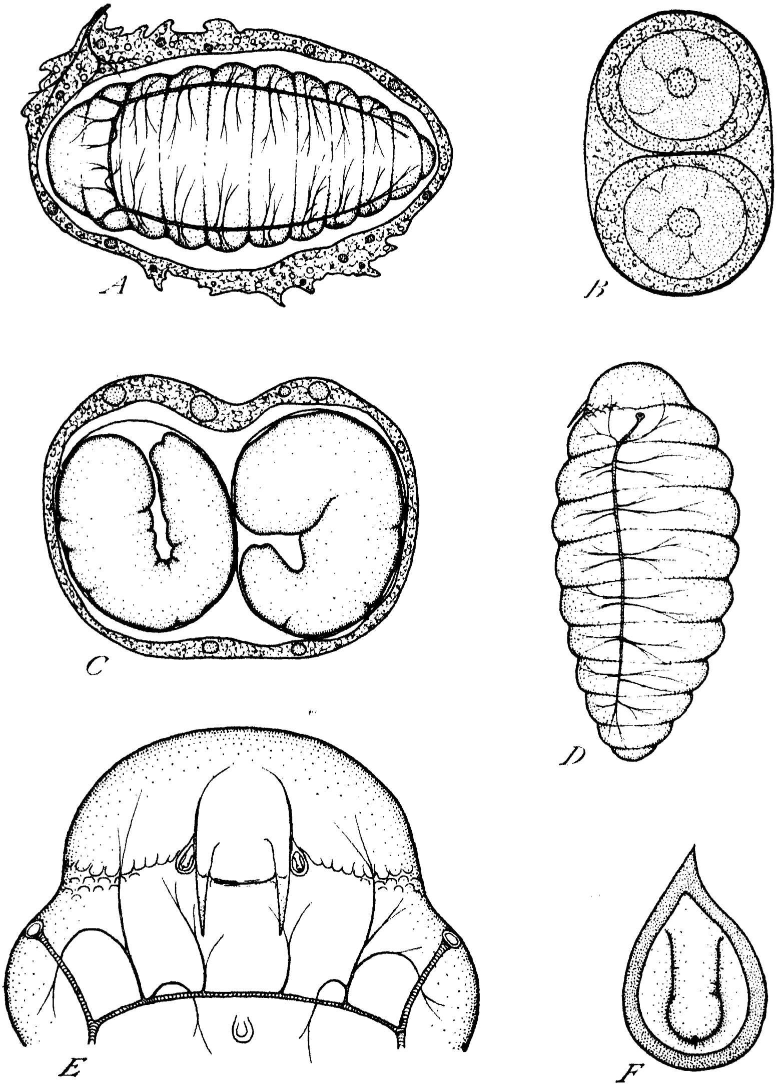


FIGURE 2.—Evidences of polyembryony and the larval morphology of *Allotropa burrelli*: *A*, Larva ready to emerge from degenerate trophamnion; *B*, twin early morulae within membrane, probably of host origin; *C*, twin embryos within a single trophamnion—definite evidence of polyembryony; *D*, mature larva; *E*, oral structures of larva; *F*, enlarged maxillary palpus.

During the fourth day the blastula attains its maximum development, with countless small cells surrounding the still faintly discernible central body (fig. 1, *G*). The parasite body has become oval and slightly irregular in outline, averaging 0.090 mm. in greatest diameter, and contains from two to five paranuclear masses of various sizes.

In the late blastula and subsequent stages it becomes increasingly difficult to dissect out the entire parasite body, since the fragile trophamnion ruptures very easily.

Although the eggs are deposited free in the host fluids, during their development most, if not all, of them eventually become associated with the mealybug fat bodies. If the fat bodies are carefully teased out and pressed beneath a cover glass, the immature stages are seen adhering to the surface or partially embedded among the fat cells. As growth proceeds and nutriment is extracted by the various trophamnia, the fat bodies become depleted of their contents until, as the embryos approach maturity, all that remains is a tangled mass of host tissues and tracheae. The earliest stage found definitely associated with the fat body was the blastula.

The tendency toward cyst formation in the Platygasteridae by those species that develop in the coelomic fluids has been well demonstrated by Marchal [6] and Leiby and Hill.[7] Other species deposit their eggs in specific organs, such as the brain, ventral nerve cord, or intestine, where nourishment is readily obtained. Although *Allotropa burrelli* does not actually become encysted, its close relation to the host fat body undoubtedly serves the same purpose.

The gastrula stage (fig. 1, *H*) appears about the fifth day after oviposition. At first a slight indentation on one side is noted, which as it grows deeper gradually becomes filled with a loose mass of proliferated cells. These cells frequently appear late in the blastula stage, and may be involved in the formation of the delicate membrane which subsequently enfolds the embryo, although occasionally they are found within this membrane. The paranuclear masses vary in number and size, and the parasite body is typically irregular in outline, measuring about 0.180 mm. long.

By the following day germ-layer formation is generally complete and organogeny has commenced (fig. 1, *I*). The parasite body now averages about 0.31 mm., the trophamnion contains a variable number of paranuclear masses, and a delicate, tight-fitting membrane surrounds the embryo. At high magnifications the cells comprising the embryo can be barely distinguished.

The embryo 7 to 8 days old (fig. 1, *J*) is considerably advanced internally and has begun to exhibit body segmentation; it is now approximately full-sized, and the trophamnion has also attained its maximum development. Henceforth the paranuclear masses become smaller and less distinct and the trophamnion becomes thinner. At this stage the parasite body measures about 0.43 mm. in length, although it varies greatly in shape. Also at this time the host fat bodies have been practically exhausted of their contents, and are nearly obscured by the gelatinous parasite bodies which adhere to one another in shapeless masses. It is extremely difficult to tease them out entire.

Just before eclosion the embryo straightens out, rupturing the inner membrane and bringing the mandibles into contact with the trophamnion. These movements probably combine to effect hatching.

---

[6] See footnote 5, p. 160.

[7] Leiby, R. W., and Hill, C. C. THE TWINNING AND MONEMBRYONIC DEVELOPMENT OF PLATYGASTER HIEMALIS, A PARASITE OF THE HESSIAN FLY. Jour. Agr. Res. 25: 337–350, illus. 1923.

The mature embryo, or unhatched larva, now averages 0.54 mm. in length (fig. 2, *A*), the parasite body having increased approximately 24 times in length. The tracheal system, however, rarely becomes filled with air, and thereby clearly distinguishable, until the trophamnion is broken.

According to all available evidence *Allotropa burrelli* normally develops monembryologically. Three twin embryos were found out of the hundreds examined. Two of these pairs were identical early morulae (fig. 2, *B*), and were complete parasite bodies, each with a separate trophamniotic layer, tightly enclosed by a thin membrane possibly of host origin; they may represent twins from a single egg deposited by accident within a fat cell or other host body, or perhaps be the result of two eggs laid simultaneously therein. The third pair (fig. 2, *C*) was more advanced, and there is no doubt that embryonic fission had actually occurred, since both embryos were surrounded by a single trophamnion. That this phenomenon is either accidental or of rare occurrence is indicated by the finding of only one such individual.

It is possible that twinning sometimes takes place at a very early stage, as has been shown by Leiby and Hill [8] in *Platygaster hiemalis* Forbes. This could be demonstrated, however, only through a detailed embryological study involving special techniques of staining and sectioning. The present study has at least shown that monembryony is the rule (additional evidence is presented in a subsequent section), and that polyembryony, if it does occur, is of an extremely simple type.

THE LARVA

Eclosion takes place from 8 to 12 days after oviposition, the average being about 9½ days, at 75°–80° F. After the trophamnion is consumned, the various host tissues and organs are attacked, until within 1 to 3 days the larva has doubled in size, average measurements being 1.10 mm. by 0.50 mm. The host is not killed until its inhabitants are approximately half-grown, although it shows little activity after the parasite larvae emerge.

The larva of *Allotropa burrelli* (fig. 2, *D*) is peculiar in several respects. It possesses but one pair of spiracles, situated on the anterolateral regions of segment 1, and is without a posterior commissure. There are 10 well-defined segments in addition to the head, which bears ventrally an unusual and apparently heretofore undescribed series of oral structures (fig. 2, *E*). The slender, amber-tipped mandibles merge imperceptibly into the head, the tips measuring 0.063 mm. in length; they are capable of only very feeble movement. Between them lies the slitlike oral aperture, which is distinguishable in life by means of the undulating labrum, while immediately adjacent on each side are the maxillary palpi. Each palpus (fig. 2, *F*) extends slightly outward from the surface, and is tipped with a microscopic dark seta. A series of minute raised protuberances traverse the posterior margin of the head from the palpi to a point near the spiracles, while the salivary duct opens centrally in the region of the anterior commissure.

The ingested food materials are contained within a peritrophic membrane which forms a complete sac. If the larva is carefully dissected, this sac floats free as a single unit; it is resilient and will bear considerable pressure beneath a cover glass before bursting.

---

[8] See footnote 7, p. 163.

That cannibalism occurs in *Allotropa burrelli* was proved by the finding of several young larvae impaled upon the mandibles of older individuals. Most of the body contents had been extracted, leaving the prey a shapeless mass distinguishable only by means of the tiny mandibles. Such examples were frequently encountered, particularly where development was uneven, the "stragglers" being eliminated in this manner. Cannibalism is apparently due to chance encounter rather than to aggressive action on the part of the parasite.

As the larvae become full-fed they lie closely packed together within the host derm. After 2 or 3 days each larva has formed about itself a parchmentlike cell or cocoon, which adheres to neighboring cocoons. The host is now rigidly distended into numerous small brownish "blisters." All attempts to locate the source of this cocoon-making material were unsuccessful; it may issue through the prominent salivary duct.

The brown, watery meconium is expelled approximately 2 days after cocoon formation, and wells up around the posterior half of the larva, where it soon hardens into an opaque layer. Larvae at this stage are identical in every respect, including oral structure, to those newly emerged or in the process of eclosion; therefore, only one instar occurs in *Allotropa burrelli.* Both *Platygaster hiemalis*[9] and *P. ornatus* Kieffer have also been shown to have but one instar.[9]

THE PUPA

Following a prepupal period of about 2 days, the pupal stage is begun. At first the eyes and then the rest of the pupa become melanized, and just prior to emergence a coal-black color is assumed. The sexes can be distinguished only by microscopic examination of the antennae. The pupal stage ranges from 11 to 16 days, with an average of about 13 days.

SEX RATIO AND NUMBER OF PARASITES PER HOST

From the original shipment from Japan, which consisted entirely of field-collected overwintering mummies, on an average 2.7 females to 1 male were obtained. Only 8.3 percent of the mummies produced an excess of males, while those yielding solely males or females were exceedingly scarce. In breeding experiments at Moorestown there were only twice as many females as males.

The material from Japan yielded 11.6 adult parasites per host, as compared with 5.2 from their progeny reared at Moorestown. The average mortality of mature larvae was also lower for the imported material—1.6 as compared with 4.3. It should be stated, however, that parasites received from Japan had been hand-picked and the imperfect and very small individuals removed. Furthermore, a portion of those propagated at Moorestown had entered the prepupal stage before being stored at 42° F. Since they overwinter as mature larvae, other stages cannot withstand low temperatures. It was also found that mealybugs parasitized in the third instar or in the early adult female stage produced the greatest number of parasites. If parasitized prior to the third instar, many hosts become mummified before attaining full growth, while those parasitized as full-fed adult females often succeed in laying eggs and can mature few if any *Allotropa* larvae. Mealybugs parasitized in the first or second instar will frequently produce solitary mummies. The number of adult parasites issuing per host has ranged from 1 to 22.

---

[9] HILL, C. C. PLATYGASTER HIEMALIS FORBES, A PARASITE OF THE HESSIAN FLY. Jour. Agr. Res. 32: 261-276, illus. 1926.

Hosts of various instars were subjected to a single oviposition by different mated females and immediately dissected to ascertain the number of eggs laid. The number ranged from 1 to 18 eggs per host, with an average of 8.8, but did not seem to depend on the size of the host. Since the number of eggs deposited is approximately equal to the number of larvae that develop, it is obvious that monembryony is the rule.

LIFE CYCLE

At temperatures in the range of 75°–80° F. the life cycle generally runs as follows, although frequent variations are found, particularly in the postembryonic stages: Egg (including parasite body) 8.0 to 12.0 days, averaging 9.5 days; larva 5.5 to 7.5 days, averaging 6.5 days; prepupa 1.5 to 2.5 days, averaging 2.0 days; pupa 11.0 to 16.0 days, averaging 13.0 days. Since considerable growth occurs during embryonic development, the incubation period is proportionally long, while the larval feeding period is comparatively short and confined to a single instar.

Mummies containing mature *Alloptropa* larvae can be stored at low temperatures for many months. The material from Japan was collected in October 1939, and upon its receipt at Moorestown was stored at 42° F. According to samples incubated at frequent intervals, emergence was wholly normal until the following June—after at least 7 months under overwintering conditions.

SUMMARY

Allotropa burrelli Mues., a gregarious platygasterine parasite of *Pseudococcus comstocki* (Kuw.), was introduced into the United States from Japan in 1939. Propagation of stocks at the Moorestown, N. J., laboratory for release in infested apple orchards afforded an opportunity for a biological study of this parasite.

The adults are small and short lived, and oviposit at random in the host body cavity. There is no preoviposition period. All nymphal stages of mealybugs are attacked, but a preference is shown for those at least half grown. Dissections have disclosed on an average 565 eggs per female.

Development is normally monembryonic, although twinning may rarely occur. The parasite body increases to approximately 24 times its initial length during incubation, nourishment being elaborated by the trophamnion with its paranuclear masses. As development proceeds the parasite body becomes closely associated with the host fat body.

There is but one instar. A single pair of spiracles is located in segment 1, and there is no posterior commissure. The mouth parts are unusual, and there are 10 body segments. The food materials are contained within a complete peritrophic sac. Supernumerary larvae and embryos are partially eliminated by cannibalistic attack.

The sex ratio has ranged from 2:1 to 3:1, with females predominating. Laboratory-reared material has produced from 1 to 22 parasites per host, with an average of 5.2. Mealybugs parasitized during the first or second stage frequently produce solitary mummies. An average of 8.8 eggs were deposited per host, regardless of size.

The life cycle ranges from 26 to 38 days, with an average of 31 days at 75°–80° F. Growth is most rapid during embryonic development, and the incubation period is nearly five times as long as the larval feeding period. Overwintering occurs as mature larvae, and mummies in that stage will endure prolonged cold storage.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., SEPTEMBER 1, 1943 NO. 5

VARIATION AND PHYSIOLOGIC SPECIALIZATION IN THE COMMON SCAB FUNGUS (ACTINOMYCES SCABIES)[1]

By L. A. SCHAAL

Associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The common scab fungus (*Actinomyces scabies* (Thaxt.) Güss.) is found in the soil of millions of acres of the best potato-growing sections of the United States, and is a source of serious economic loss to growers. Seed and soil treatments have been devised, but these are expensive and often unsatisfactory. The most effective means of controlling scab is to breed varieties that are resistant to scab. This method frequently involves the expenditure of considerable time and money, but the results when once obtained appear to be permanent.

Clark, Stevenson, and Schaal (*3*)[2] and Krantz and Eide (*6*) have shown that there are wide differences among varieties and even among seedlings in their reaction to scab. Some are nearly immune, others completely susceptible. It has also been shown that immunity and susceptibility are transmissible to the progeny.

In breeding new varieties that combine scab resistance with high yield and good cooking and market qualities it has been found that a variety which is resistant to scab in one locality may be less resistant in another. In a series of tests made in a number of potato scab gardens in different parts of the United States (*14*), it was found that the variability was due in part to environmental conditions and in part to physiologic races of *Actinomyces scabies*. That *A. scabies* may comprise different strains was considered by Millard and Burr (*10*), and the existence of races was established by Leach, Decker, and Becker (*7*). The present work was undertaken to study (1) the occurrence of physiologic races of *A. scabies* and their relation to the variable behavior of scab, (2) the factors affecting the development of races or strains, and (3) the necessity for testing the reaction of potato varieties to the various parasitic strains of the scab organism. The studies were started at the Aroostook Farm, Presque Isle, Maine, and were continued at the Plant Industry Station, Beltsville, Md., and later at University Farm, St. Paul, Minn.

---

[1] Received for publication April 26, 1943. Assistance was furnished by the personnel of the Works Projects Administration, official project No. 65-1-71-140, sponsored by the University of Minnesota. The author is indebted to E. C. Stakman, of the University of Minnesota, for advice and criticism throughout the investigation and to F. J. Stevenson and E. S. Schultz, of the Division of Fruit and Vegetable Crops and Diseases, for certain plant materials.

[2] Italic numbers in parentheses refer to Literature Cited, p. 186.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 5
Sept. 1, 1944
Key No. G-1314

METHODS OF ISOLATION AND DESIGNATION OF CULTURES

The method of isolating *Actinomyces scabies* from deep pustules on the tubers of potatoes (*Solanum tuberosum* L.) was a slight modification of that described by Shapovalov (*13*). An infected tuber was washed, the pustules were wiped lightly with cotton saturated with 95 percent alcohol, and the alcohol on the surface was burned. The corky covering was then lifted with a sterile scalpel, and the underlying tuber tissue was transferred to a tube of melted agar; transfers were made to other tubes of melted agar, which were poured into Petri dishes in order to make it easier to obtain colonies of *A. scabies* free from other fungi and bacteria. When isolates were made from shallow or superficial scab spots the whole scabby area was flamed lightly, macerated, and transferred to melted agar. Dilution plates were then poured, as described above. In most cases colonies of *A. scabies* appeared after 48 hours.

The scab organism was isolated from soil by pouring dilution plates from 10-gm. soil samples.

Single-cell isolates were obtained by using a micromanipulator with a research microscope. With the end of a needle a small quantity of aerial mycelium was tapped lightly over a sterile cover slip; on the surface of this were deposited a number of single cells. The cover slip was then inverted over a modified Van Tieghem cell for examination under the microscope. By means of a glass rod drawn to a diameter of 1μ or 2μ single cells were picked up and transferred to an agar drop on a cover slip. The cover slip was then inverted over a Van Tieghem cell containing sterile water and placed in an incubator. If the cell germinated, it was transferred to an agar slant from which subcultures were made later.

The following system was used to designate the cultures: M1, M2, etc., are single-cell cultures; S1, S2, etc., are sectors from such cultures. For example, 147M1S2 is sector 2 of single-cell culture M1 of parent isolate 147. Single-cell cultures from sector cultures were in turn indicated by M1, M2, etc.; for example, 147M6S3M1.

SOURCE AND GENERAL CHARACTERISTICS OF ISOLATES

Waksman (*15*) described a number of species of *Actinomyces* on the basis of cultural characters, but he did not give a definite specific name to the strains found on potatoes. Lutman and Cunningham (*8*) concluded that all known strains were able to produce a brown pigmentation on media containing proteins. The isolates of *Actinomyces scabies* listed in table 1 were obtained from scab pustules on potato tubers grown in various States. Scab cultures were also obtained from Ireland and Australia. In the early studies it was noted that sometimes several culturally different isolates of *A. scabies* could be obtained from a single pustule. Cultures from these colonies differed in growth type and in the pigmentation that they produced on a given medium. Usually isolates of a given cultural type were consistently obtained from tubers grown in a certain area or on a certain soil type.

Table 2 lists the colors of the mycelium and of the pigment produced by 33 cultures when grown on the modified potato-dextrose agar designated as medium 1 in table 3. All cultures listed in table 2 were found to be pathogenic, but they varied in degree of virulence, as indicated in table 1.

TABLE 1.—*Source of isolates and cultures of Actinomyces scabies, type of pustule, and most susceptible of tested potato varieties*

Isolate or culture No.	Source of infected tuber or culture	Pustule type[1] on—		Soil type on which naturally infected tuber grew	Most susceptible variety tested
		Naturally infected tuber	Inoculated tuber		
J11M1	Minnesota	1	R	Mineral	Katahdin.
23M1	Maine	1	1	do	Green Mountain.
66M1	do	2	2	do	Do.
104[2,3]	Ireland		1		Do.
105[2]	Australia		1		Do.
108M1	Minnesota	2	2	Peat	Irish Cobbler.
110M1	do	1	1	do	Do.
115M1	do	1	2	Mineral	Do.
117M2	Colorado	1	R	do	Katahdin.[4]
118M4	Minnesota	1	1	do	Green Mountain.
119M1	Colorado	1	1	do	Katahdin.
124M2	Minnesota	1	R	Peat	U. S. D. A. seedling.
125M1	do	1	1	do	Green Mountain.
126M2	do	1	1	do	Do.
133M1	Colorado	1	1	Mineral	Do.
136M3	Minnesota	1	1	Peat	Do.
143M1	Colorado	1	1	Mineral	Katahdin.
147M2	Minnesota	2	2	do	Do.
153M1	do	1	1	do	Green Mountain.
166M1	Colorado	R	R	do	U. S. D. A. seedling.
176M4	Wyoming	1	1	do	Green Mountain.
181M2	do	1	1	do	Do.
183M1	do	1	2	do	Do.
185M1	Minnesota	2	2	Peat	U. S. D. A. seedling.
200M1	Colorado	3	R	Mineral	Do.
205M3	Minnesota	1	1	do	Do.
210M1	do	1	1	do	Katahdin.
220M2	do	1	2	do	U. S. D. A. seedling.
223M2	do	1	1	Peat	Green Mountain.
234M1	Maine	1	1	Mineral	Do.
242M3	California	1	1	do	Do.
250M1	Minnesota	1	1	Peat	Irish Cobbler.
252M3	do	2	1	do	Do.

[1] 1, susceptible; 2, moderately susceptible; 3, resistant; R, russeting caused by scab organism (skin checked and rough).

[2] Not single-spored.

[3] From T. N. Greeves, Queens University, Belfast.

[4] Only variety tested.

TABLE 2.—*Color characteristics of thirty-three 6-week-old pathogenic isolates and cultures of Actinomyces scabies grown on modified potato-dextrose agar*

Isolate or culture No.	Mycelium	Pigment in agar	Isolate or culture No.	Mycelium	Pigment in agar
J11M1	Yellow	Deep yellow.	143M1	Blue gray	Dark brown.
23M1	Gray	None.	147M2	Light blue	Deep blue.
66M1	do	Do.	153M1	Green gray	Brown.
104[1]	Green	Blue gray.	166M1	Blue gray	None.
105[1]	Gray	None.	176M4	Light blue	Violet to red.
108M1	Light gray	Do.	181M2	Dark gray	Dark brown.
110M1	Blue gray	Brown.	183M1	Light gray	Brown.
115M1	Dark gray	Do.	185M1	Blue gray	Pink to red.
117M2	Buff[2]	Light brown.	200M1	Dark gray	Brown.
118M4	Dark gray	Dark brown.	205M3	Buff	Light brown.
119M1	do	None.	210M1	Gray	None.
124M2	do	Pink, turning blue as culture aged.	220M2	Red[3]	Dark red.
			223M2	White	None.
125M1	Light gray	Brown.	234M1	Gray	Light brown.
126M2	Blue gray	None.	242M3	Dark gray	Brown.
133M1	Dark gray	Dark brown.	250M1	Light gray	None.
136M3	Light gray	Do.	252M3	do	Brown.

[1] Not single-spored. [2] Aerial. [3] Cheesy.

TABLE 3.—*Media used in studying cultural characters of Actinomyces scabies*

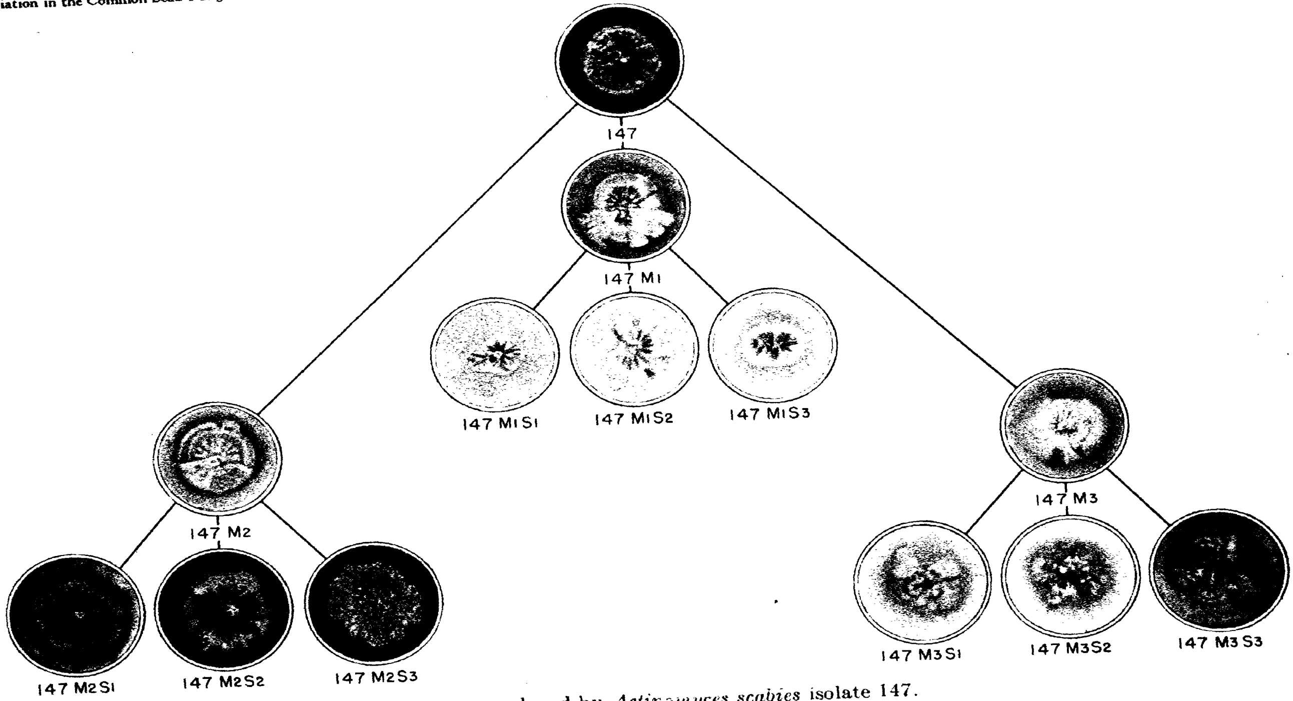
Medium			pH afte sterilizati
No.	Name	Formula	
1	Modified potato-dextrose agar.	Peeled potatoes, 300 gm Dextrose, 5 gm Agar, 20 gm Tap water, 1,000 cc Adjusted to pH 6.8	6.8 to 7.
2	Egg-albumen agar (Waksman).	Dextrose, 10 gm KH_2PO_4, 0.5 gm $MgSO_4$, 0.2 gm $Fe_2(SO_4)_3$, trace Egg albumen, 0.15 gm Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.5 to 7.
3	Bacto-nutrient agar	Dehydrated Bacto-nutrient agar, 23 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8
4	Richards' agar (modified).	KNO_3, 1 gm KH_2PO_4, 0.5 gm $MgSO_4$, 0.2 gm $FeCl_3$, trace Sucrose, 5.0 gm Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8 to 7.
5	Richards' agar (modified) plus thiamine.	Same as 4 with 0.3 gm. thiamine added Adjusted to pH 7.0	6.8 to 7.
6	Richards' agar (modified).	$MgSO_4$, 1.0 gm Urea, 2.0 gm KH_2PO_4, 1.0 gm $FeCl_3$, trace Agar, 20.0 gm Distilled water, 1,000 cc Adjusted to pH 7.0	6.8 to 7.
7	Potato-dextrose agar	Same as 1 except that whole tubers of a potato variety resistant to scab were used as a source of potato juice.	6.8 to 7.
8	do	Same as 7 except that potato juice was from whole tubers of a scab-susceptible variety (Irish Cobbler).	6.8 to 7.

CULTURAL VARIATION

FREQUENCY OF SECTORING

Sectoring in *Actinomyces scabies*, which was first observed in 1-month-old or older cultures in flasks, has not previously been reported in the literature. Several culturally different strains produced sectors that differed greatly from one another. Culture 147M1, isolated from a pustule on a tuber grown at Castle Danger, Minn., produced many sectors during 4 weeks in culture. In some cases as many as nine sectors appeared in a single colony; these varied in type and rate of mycelial growth and in color of pigment. Other cultures sectored less frequently.

Plate 1 shows diagrammatically the relation of the culturally different strains 147M1, 147M2, and 147M3, which originated as single cells from the parent isolate 147. The parent isolate 147 (pl. 1) had a deep-blue pigment, but only one of the single cells isolated from it produced the deep-blue pigment or lighter shades of blue. Culture 147M1 developed sectors. Cultures from three of these failed to produce the deep-blue pigment in the medium. After 3 months cultures from eight sectors of 147M1 had produced sectors, and a culture from one sector had failed to produce sectors. Apparently this sector was stable since further culturing in media of various types and with different pH values failed to induce sectoring. Although it might have



Variants produced by *Actinomyces scabies* isolate 147.

been possible to obtain several more sectors from each culture, cultures were not made from all sectors. Several of the sectors shown in cultures 147M3S2 and 147M3S3 were cultured later, and these in turn formed sectors. This process could have been repeated, and many culturally different strains of *Actinomyces scabies* could probably have been produced.

Figure 1 shows diagrammatically how sectoring occurred in isolate 66. In this case, too, some of the variants sectored. Neither the parent nor the variates produced pigment in the medium. Sectoring of the type found in certain cultures of *Actinomyces scabies* might account for the many strains of the fungus that have been found in soils and on potato tubers.

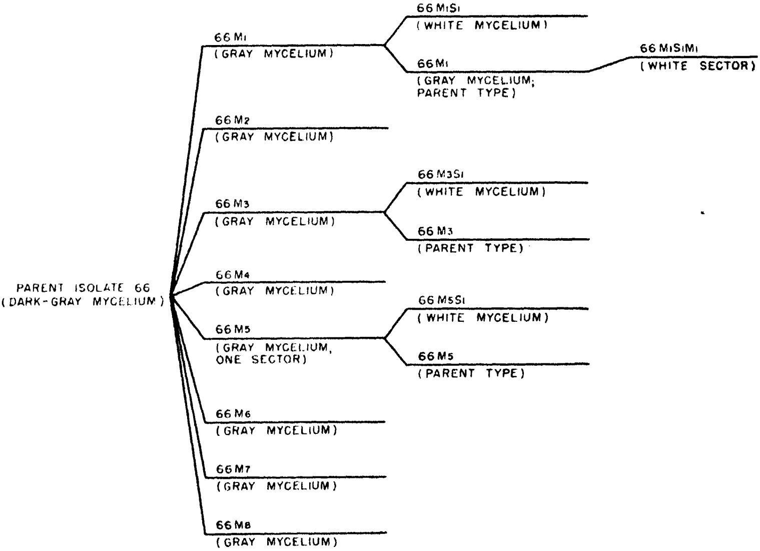


FIGURE 1.—Diagrammatic relation of the sectors produced by three of eight single-cell cultures originating from parent isolate 66.

MORPHOLOGY OF VARIOUS ISOLATES

Studies were made to determine how culturally different isolates would vary morphologically. In these studies mycelial cells rather than spores were used for measurement, since some cultures did not produce any aerial spores or spirals on any medium used. The size of mycelial cells and the presence or absence of spirals on the aerial mycelium were noted.

Drechsler (*4*) found that many species of *Actinomyces* could be differentiated on the basis of morphology, that the presence or absence of spirals constituted a differential character, that some of the spirals were sinistrorse and others dextrorse, and that there were noticeable differences in the sizes of mycelial cells of the several species listed.

The mean sizes of cells in various cultures differed. The cells of the spirals were sometimes twice as large as the mycelial cells, and several cultures produced larger cells on the tips of the spirals. Some investigators speak of these terminal cells as spores.

Table 4 gives the mean sizes of mycelial cells and records the presence or absence of spirals in 20 cultures of *Actinomyces scabies* grown on modified potato-dextrose, egg-albumen, and Bacto-nutrient agars. The cells used for the measurements were taken near the center of the colony, and the means of 100 cells were obtained. Six of the cultures did not produce spirals on any of the media; 14 produced sinistrorse or dextrorse spirals in varying abundance. Both the type of spiral and the direction of the turns appeared to vary with the culture. Culture 166M2 produced abundant aerial mycelium without spirals, and culture 220M1 produced a slimy growth without spirals. Culture 23M1 produced spirals, whereas 23M1S1M1, a single-cell culture from a sector from 23M1, did not produce spirals. There was very little variation in the sizes of cells in most cultures. The parent culture 23M1 had mycelial cells 1μ by 2μ, and those of its variant measured 1μ by 1μ. This difference in cell size indicates that when sectoring occurs in *A. scabies* the morphology of the variant may differ from that of the parent.

Attempts to demonstrate the presence of nuclei in *Actinomyces scabies* failed. Feulgen's stain was found best for staining the cells, but even with this, nuclei could not be recognized with certainty. There may, however, be numerous nuclei in a single cell, for the cell contents under high magnification appear to contain many dark-staining, minute objects.

TABLE 4.—*Cell sizes and presence or absence of spirals in cultures of Actinomyces scabies on different media*[1]

Culture No.	Medium 1 (modified potato-dextrose agar)		Medium 2 (egg-albumen agar)		Medium 3 (Bacto-nutrient agar)	
	Spirals present (+) or absent (−)	Mean size of cells	Spirals present (+) or absent (−)	Mean size of cells	Spirals present (+) or absent (−)	Mean size of cells
		μ		μ		μ
23M1	+	1×2	+	1×2	+	1×2
23M1S1M1	−	1×1	−	1×1	−	1×1
66M1	+	1.5×2	+	1.5×2	+	1.5×2
104M1	+	1.5×2	+	1.5×2	+	1.5×2
105M1	+	1×1	+	1×1	+	1×1
119M1	−	1.5×2	−	1.5×2	−	1.5×2
133M1	+	1×2	+	1×2	+	1×2
136M4	+	2×2	+	1.5×2	+	2×2
147M1	+	2×2	+	2×2	+	2×2
147M1S1	+	2×2	+	2×2	+	2×2
147M6S3M1	+	1×2	+	1×2	+	1×2
153M1	+	1.5×2	+	1.5×2	+	1.5×2
157M1	+	2×2	+	1.5×2	+	1.5×2
166M2	−	1×1	−	1×1	−	1×1
176M4	+	1.5×2	+	1.5×2.5	+	1.5×2
181M1	+	1.5×2	+	1.5×2	+	1.5×2
200M1	−	2×2	−	2×2	−	2×2
220M1	−	1×1.5	−	1×1.5	−	1×1
234M1	+	1.5×1.5	+	1.5×1.5	+	1.5×1.5
250M2	−	1×2	−	1×2	−	1×2

[1] See table 3 for formulas.

FACTORS AFFECTING GROWTH AND SECTORING

EFFECTS OF NUTRIENTS

The effects of different nutrients on the rate of growth and the sectoring of *Actinomyces scabies* were studied by using the synthetic media 2, 3, 4, 5, and 6 described in table 3. All these media were adjusted to pH 7.0 before sterilization; after sterilization the pH values varied from 6.5 to 7.0. Barss (*2*) called attention to the variable composition of media made from plant or animal tissue and the difficulty of using such media to study rate and character of growth. This difficulty is especially great in a study of the scab organism, and for that reason synthetic media were used.

Ten different cultures of *Actinomyces scabies* were grown in duplicate on the five synthetic and modified potato-dextrose media in 250-cc. Erlenmeyer flasks at approximately 20° C. for 8 weeks. The mycelial growth was more rapid on the synthetic media than on the potato-dextrose agar adjusted to the same pH value, but aerial mycelia were sparse and could be seen only by careful observation. The most rapid growth was made on medium 5, which contained 0.3 mg. of thiamine per liter. Best growth of aerial mycelium was noted on medium 3. A medium having a high nitrogen content inhibited aerial mycelium and limited the size of the colonies. Afanasiev (*1*) found that high nitrogen inhibited production of aerial mycelium by parasitic races of *A. scabies* but did not affect the saprophytes; this finding partly substantiates the present results.

After 6 weeks only four cultures showed sectors, but after 8 weeks all had produced them. At the end of 8 weeks the greatest number of sectors had been produced on medium 5. The stimulation of the rate of mycelial growth and of the production of sectors by the addition of thiamine agrees with the results of Oyama (*11*), who found that the vitamin B complex increased growth in the Dermatocytes.

EFFECTS OF TEMPERATURE

Cultures of four strains of *Actinomyces* isolated from tubers from three States, representing different mean seasonable temperatures, were grown for 56 days on modified potato-dextrose agar adjusted to pH 6.8 at 18°, 20°, 22°, and 24° C.

The differences in temperature tolerance of these four cultures were not great, as the growth curves of figure 2 show. All the cultures grew readily at temperatures from 18° to 24° C. As the colonies increased beyond a diameter of 60 mm. the rate of growth became slower. Regardless of how long a culture was grown in a 250-cc. flask containing 75 cc. of medium, the diameter of the colony never exceeded 65 of the 75 mm. of surface diameter available.

No consistent differences appeared in the rate of sectoring and the type and number of sectors produced by the four cultures grown at 18°, 20°, 22°, and 24° C. over a period of 12 weeks. In several duplicate cultures sectors developing at 22° and 24° were almost identical in appearance, whereas at 18° and 20° differences in number and type of sectors were more evident; at 18° sectors were present in several of the cultures, but they were smaller than those in cultures grown at 22° and 24°.

EFFECTS OF HYDROGEN-ION CONCENTRATION

It has been commonly accepted that *Actinomyces scabies* will not grow well on acid media and that scab infection does not occur on potato tubers growing in soil with a pH value less than 5. However, cases have been reported (*12*) where serious scab has occurred in soil of pH 5.4.

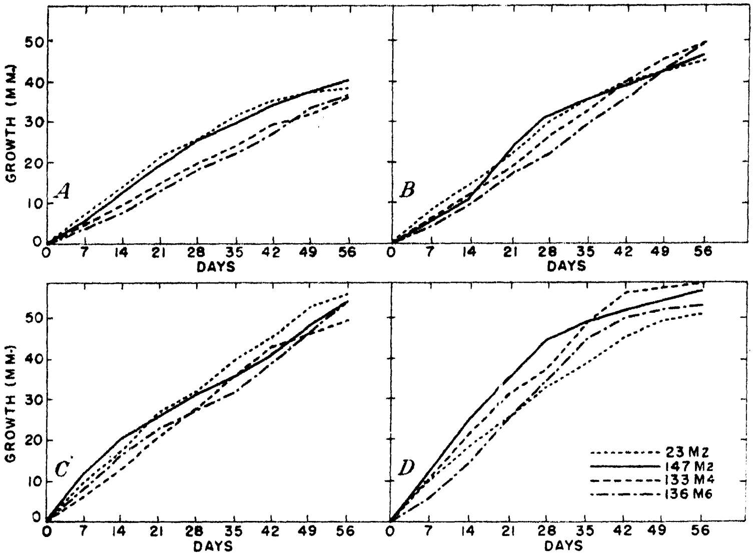


FIGURE 2.—Growth of four cultures of *Actinomyces scabies* on potato-dextrose agar adjusted to pH 6.8: *A*, 18°; *B*, 20°; *C*, 22°; and *D*, 24° C.

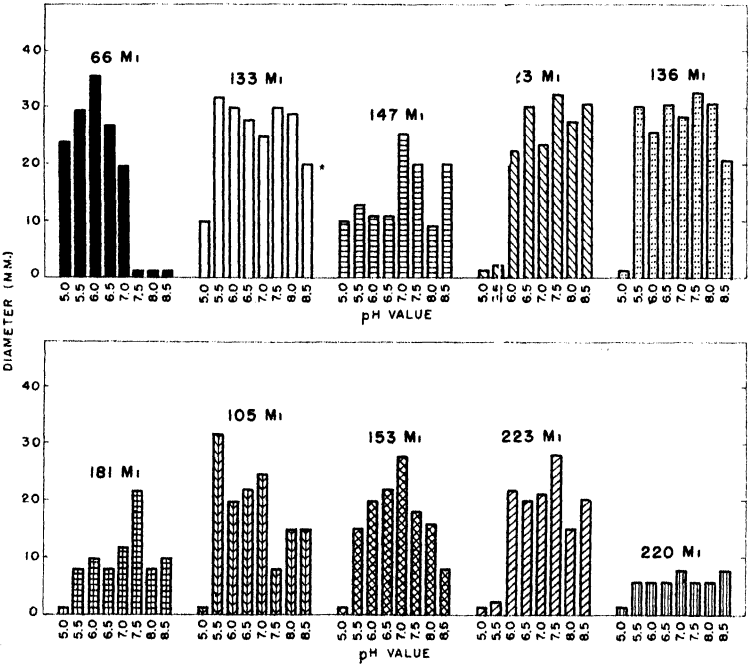


FIGURE 3.—Growth of 10 *Actinomyces scabies* cultures after 7 days on potato-dextrose agar adjusted to different pH values.

Ten cultures of *Actinomyces scabies* obtained from different potato-growing areas were grown on modified potato-dextrose agar adjusted to 8 different pH values from 5.0 to 8.5 in duplicate 250-cc. flasks containing 75 cc. of medium. All flasks were inoculated uniformly by placing a drop of sterile water containing a high concentration of spores in the center of the medium and were incubated at room temperature (approximately 22° C.). The diameters of the colonies were measured at the end of 7 and 60 days. Figures 3 and 4 show the relative growths of the 10 cultures.

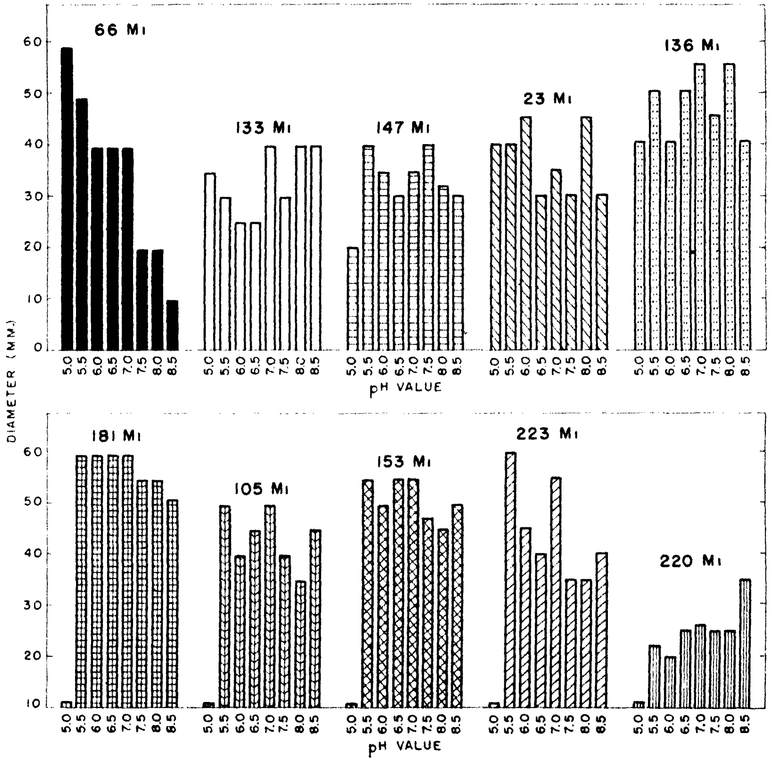


FIGURE 4.—Growth of 10 *Actinomyces scabies* cultures after 60 days on potato-dextrose agar adjusted to different pH values.

After 7 days seven of the cultures showed very little growth at pH 5, but four of these seven developed rapidly at pH 5.5, indicating that their pH tolerance was between 5.0 and 5.5 (fig. 3). Culture 220M1 grew slowly on all media; and there was little difference in its rate of growth regardless of the pH of the medium. Maximum growth occurred at pH 8.5 (fig. 4), but subsequent tests with this culture showed that it did not grow more even on more alkaline media.

These observations indicate that many strains of *Actinomyces scabies* grow best at approximately pH 7.0 and do poorly below 5.5.

This finding agrees with observations on the effect of the hydrogen-ion concentration of the soil on scab severity. At the end of 60 days, however, culture 66M1 had made its maximum growth at pH 5.0 (fig. 4). This culture, which was isolated from a tuber grown in soil where scab was severe, could not be distinguished culturally or morphologically from certain others, but it could be differentiated by its tolerance to acidity and its parasitism on certain potato varieties (*12*).

While these cultures were growing in flasks, observations were also made on the effect of different hydrogen-ion concentrations on sectoring. At the end of 40 days very few sectors had appeared, but at the end of 60 days sectors were developing in one or both of the duplicate flasks that showed appreciable growth. Sectors were noted at all pH values above 5.0, but no single type predominated. Variation in *Actinomyces scabies* apparently is not greatly affected by the reaction of the supporting medium.

GREENHOUSE TESTS OF PATHOGENICITY

METHODS OF TESTING

The method used to distinguish parasitically different strains consisted in growing different varieties of potatoes in the greenhouse and inoculating the soil with cultures of *Actinomyces scabies* immediately after the tubers were planted.

In the inoculation studies in the greenhouse at Beltsville, Md., and at St. Paul, Minn., seed pieces were treated with a 1 to 1,000 mercuric chloride solution for 1½ hours, then washed under running water for 10 minutes, planted in soil with a pH favorable for the development of scab, and steam-sterilized for 10 hours at 10 pounds' pressure. The inoculum was added to the soil as a water suspension of the scab organism. The concentration of the inoculum was kept as uniform as possible for the several tests. When a large amount of sterilized soil was inoculated and placed in the greenhouse bench, the inoculum was added with a sprinkling can as the soil was being mixed.

When large quantities of inoculum were needed a spore suspension was made in sterile distilled water and several drops were spread over the surface of agar medium in Petri dishes. After considerable aerial mycelium had been produced it was scraped off with a sterile scalpel and placed in sterile tap water; measured quantities of this suspension were then added to the soil.

All pot inoculations were made in duplicate 6-inch pots, which were separated on the bench by panes of glass high enough to prevent the mixing of cultures by splashing when the pots were watered. Soil moisture was kept as uniform as possible. Preliminary tests showed that ordinary tap water did not contain *Actinomyces;* hence it was not necessary to use sterile water. All plants were allowed to mature, and notes were taken when the tubers were dug.

Pustule type was considered the most important factor in determining the degree of parasitism of the different isolates. The number of pustules produced on a tuber was not considered as important as whether the infection was deep, shallow, or russeted. In all cases there were sufficient pustules of a given type on the tubers to make accurate readings possible. A modification of the system of estimating scab infection employed by Clark, Stevenson, and Schaal (*3*) was used; according to this system 1 indicates susceptibility and 3 resistance. This system was found to give a satisfactory differentiation between the types of infection. The type of scab that caused a normally smooth variety of potato to look russeted and scurfy was called russeting, as distinguished from the pustule type.

The commercial and seedling varieties of potatoes tested to determine whether the various isolates constitute physiological races of the scab organism are listed in table 5. Commercial varieties are designated by names and seedling varieties by numbers.

TABLE 5.—*Reaction of certain commercial varieties and seedlings of potatoes grown in a greenhouse to single-cell cultures of Actinomyces scabies*

Culture No.	Source of infected tuber or culture	Pustule type[1] produced on tubers of—							
		Katahdin	Green Mountain	Seedling 1037-5	Seedling 295-12	Seedling 627-213	Seedling 528-212	Hindenburg	Seedling 2621
M5M1	Michigan[2]	0	3	0	0	0	R	3	0
J11M1	Minnesota	0	0	0	0	0	0	0	0
23M1	Maine	1	1	2	2	1	2	3	3
23M1S1	do	2	1	2	2	2	2	0	0
66M1	do	2	2	1	3	2	3	0	3
66M1S1	do	2	R	R	0	0	R	0	0
104M1	Ireland	3	1	2	0	0	3	0	0
105M1	Australia	1	1	0	0	0	0	0	0
110M1	Minnesota	1	1	1	2		2	3	
118M1	do	3	1	2	3	0	3		
119M1	Colorado	1	2	0	3	R	R		
124M1	Minnesota	0	R	0	R		R		
128M1	Colorado	3	R	2	0	0	0	0	
133M1	do	1	1	1	2	3			3
136M1	Minnesota	1	1	1	2	0	3	0	0
143M1	Colorado	1	1	2	1	3	2	0	
147M1	Minnesota	0	2	1	R		3	0	1
153M1	do	3	1	1	0	0	0	0	1
157M1	Colorado	1	1	1	2		0	3	
166M1	do	2	3	R	0	0	3	0	0
170M1	do	0	0	3	0	3	3	0	
176M1	Wyoming	0	1	2	0	0	0	0	0
178M1	do	0	1	2	0	2	R	0	
181M1	do	1	1	1	1	2	2	3	1
183M1	do	3	1	1	0		3	3	0
194M1	Colorado	1	0	1	0	0	0	0	0
200M1	do	1	1	2	0	0	2	0	0
205M1	Minnesota	2	0	1	2	2	2	0	3
210M1	do	1	1	3	R	0	3	0	1
217M1	do	1	1	1	R	3	2	0	1
220M1	do	0	0	0	2	0	0	0	0
223M1	do	1	1	1	3	1	3	0	1
231M1	do	0	1	0	0	3	0	0	2
232M1	do	0	3	0	0	0	3	0	3
236M1	California	2	1	2	R	0	2	0	0
242M1	do	0	1	3	3	0	0	0	0
250M1	Minnesota	0	1	2	0	0	0	0	0
252M1	do	2	1	1	R	0	3	0	1
3031M1	New Jersey[3]	0	3	2		0	0		
3352M1	do[3]	0	3	2	2	0	0		

[1] 0, no scab; 1, susceptible; 2, moderately susceptible; 3, resistant; R, russeting caused by scab organism (skin checked and rough).

[2] From J. H. Muncie, Michigan Agricultural Experiment Station.

[3] From New Jersey Agricultural Experiment Station.

RELATIVE PATHOGENICITY OF VARIOUS CULTURES

Table 5 summarizes the results of 2 inoculation tests to determine the scab reaction of 3 commercial and 5 seedling varieties of potatoes selected for their differential reaction, as noted in field experiments, to 40 cultures of *Actinomyces scabies*. All but 1 of the cultures were found to be pathogenic in varying degrees, and even this was parasitic in other tests. These experiments show that when a single-cell culture is used shortly after isolation from a tuber as inoculum for tubers grown in sterilized soil, it produces a distinct pustule type on a given variety of potato. In the field more than 1 type of pustule may be found on a tuber. This suggests the presence of more than 1 parasitic strain in the soil. The scab reactions of 2 commercial and 4 seedling varieties to 11 isolates are shown in figure 5. Plainly these isolates differed in pathogenicity as well as in cultural and physiological characters.

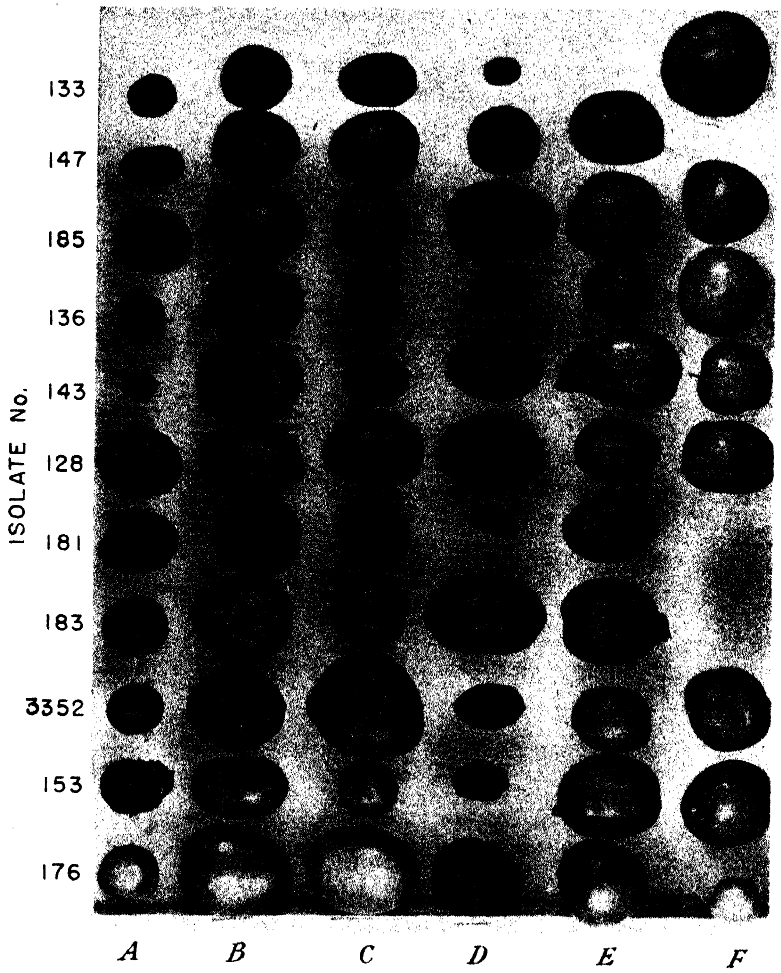


Figure 5.—Representative types of scab infection produced by 11 isolates of *Actinomyces scabies* (designated by numbers at left) on commercial and seedling varieties of potatoes in greenhouse inoculation tests: *A*, Green Mountain; *B*, seedling 295–12; *C*, Katahdin; *D*, seedling 1037–5; *E*, seedling 528–212; and *F*, seedling 627–213.

RELATIVE PATHOGENICITY OF VARIANT AND PARENT CULTURES

The occurrence of cultural variants suggested that differences in pathogenicity might exist. Consequently, tests were made to see whether such differences really occurred. Cultures 23M1S1 and 66M1S1 were culturally different from their parent cultures. Pathogenicity tests of these variants and the parent cultures showed slight differences in the type of pustules produced on some of the varieties (table 5). These variants were less virulent than the parent cultures on some varieties. For example, culture 66M1S1 produced only russeting-type scab on Green Mountain, whereas the parent culture (66M1) produced type 2 pustules. Parent culture 66M1 was more parasitic than the variant on all varieties tested except Katahdin and Hindenburg. Parent culture 23M1 was more parasitic than the variant 23M1S1 on Katahdin, Hindenburg, and seedling varieties 627–213 and 2621. On the other varieties tested this variant produced pustules similar to those caused by the parent culture. The results of these limited tests indicate that the variants produced by parasitic cultures of *Actinomyces scabies* may differ in pathogenicity from the parent cultures.

RELATION OF VARIOUS ISOLATES TO RUSSETING

Russeting of normally smooth-skinned varieties of potatoes has been attributed to various causes. Many growers are of the opinion that it is caused by physical and chemical conditions of the soil, such as high acidity or high alkalinity, or by fertilizer burn. Lutman, Livingston, and Schmidt (*9*) suggested that pathogenicity might be reduced if the organism existed for a time in the absence of the potato and that it would then produce a russeting or milder form of scab. Goss (*5*) suggested that russeting in Green Mountain under field conditions may be caused by a different species of *Actinomyces*.

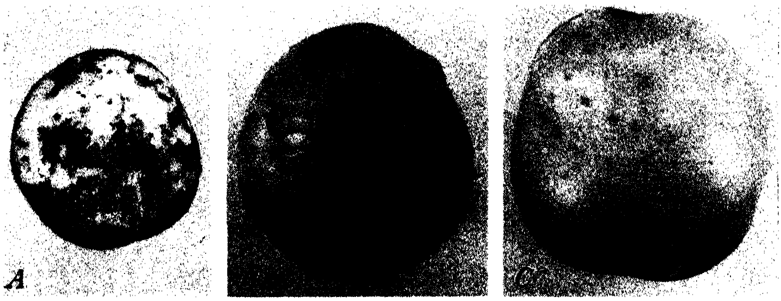


FIGURE 6.—*A*, Russeting type of scab on a Green Mountain tuber grown in Maine; *B*, russeting on Green Mountain tuber inoculated with *Actinomyces* 124M1; *C*, Green Mountain tuber grown in uninoculated soil.

The data presented in table 5 show that one culture, 124M1, when it caused infection, produced russeting only. Another, 166M1, produced only shallow type 2 or 3 pustules or russeting on the varieties that became infected. Isolate 166 was from a tuber showing russet-type infection. Of the 40 cultures listed in table 5, 12 produced russeting on 1 or more of the varieties tested, some of which are normally smooth. Figure 6, *B*, shows typical russeting produced in greenhouse inoculations by culture 124M1. Reisolates from this type of scab appeared identical with the inoculum. Russeting of the type shown in figure 6, *B*, can be caused by certain strains of *Actinomyces scabies* on normally smooth-skinned potato tubers.

VARIETAL REACTION TO SCAB IN DIFFERENT LOCALITIES

In breeding potatoes for resistance to scab and various other diseases the possibility of physiologic specialization of the pathogen must be considered. In uniform scab test plots in various potato-growing areas it has been found that a given variety may differ greatly in its reaction to scab in different localities (figs. 7 to 10). The pronounced differences in the types of pustules indicate, as the greenhouse tests did, that different strains or races of *Actinomyces scabies* may be involved.

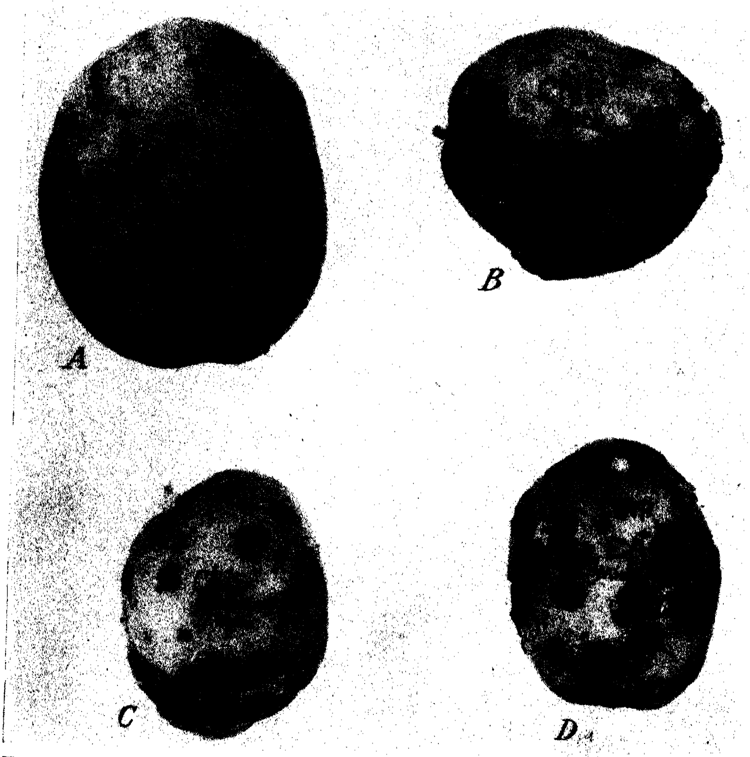


FIGURE 7.—Types of scab pustules on Chippewa tubers produced in scab test plots in Rhode Island (*A*); Michigan (*B*); Colorado (*C*); and Minnesota (*D*). In Michigan and Minnesota the plots were on peat soil; in Rhode Island and Colorado on mineral soil.

DISCUSSION

In these studies an attempt was made to determine the existence and possible importance of strains and physiologic races of *Actinomyces*

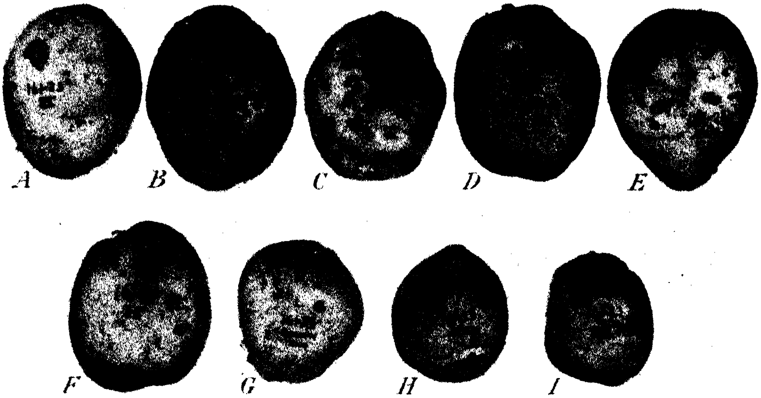


FIGURE 8.—Types of scab pustules on tubers of United States Department of Agriculture seedling 161–23 produced in scab test plots in Rhode Island (*A*); Michigan (*B*); Maine (*C*); New York (*D*); Avon, Colo. (*E*); Iowa (*F*); Greeley, Colo. (*G*); Indiana (*H*); and Minnesota (*I*). In Iowa, Michigan, and Minnesota the plots were on peat soil; in the other States on mineral soil.

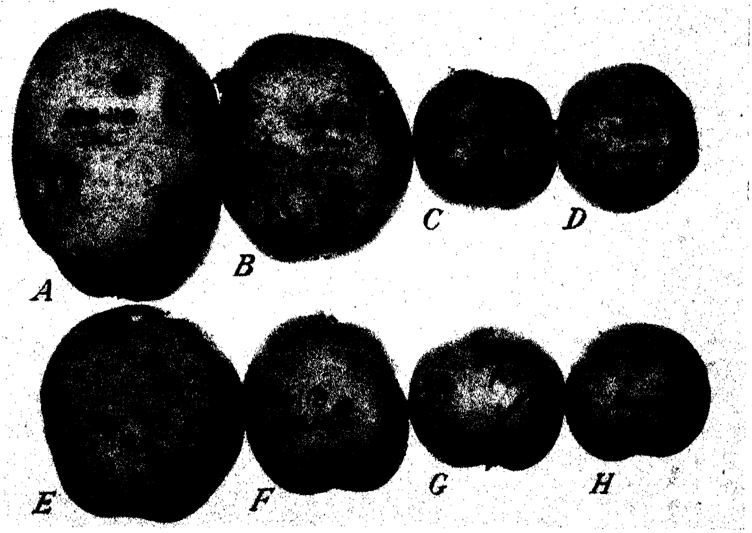


FIGURE 9.—Type of scab pustules on tubers of United States Department of Agriculture seedling 528–118 produced in scab test plots in Indiana (*A*); Colorado (*B*); Maine (*C*); Michigan (*D*); Rhode Island (*E*); Minnesota (*F*); New York (*G*); and Iowa (*H*). This seedling was resistant to scab in these eight States.

scabies. That strains do exist has been well established by these studies. From a collection of scabby tubers from different potato-growing areas it was possible to obtain isolates of *A. scabies* differing greatly in type and color of mycelium and in color of pigment produced in the medium. It seemed possible that these strains might differ also in morphological characters and degree of pathogenicity, as experiments by other investigators had suggested that parasitic strains of *A. scabies* might have certain cultural characters in common and that it might be possible to distinguish parasitic and saprophytic types by simpler and easier methods than by inoculation of tubers, which is always a laborious and slow method. In these studies, however, no correlation was detected between degree of parasitism and degree of color of mycelium or medium. There were no morphological characters of *A. scabies* that distinguished parasitic from nonparasitic strains. The production of spirals on the aerial mycelium appears to be constant for a given strain, and the medium on which the culture was grown did not affect this characteristic. Both spiral- and nonspiral-producing strains were found to be parasitic on potato tubers; the direction of the turns in the spirals was not correlated with pathogenicity. The size of mycelial cells did not serve as a distinguishing character. In short, inoculation of potato tubers seems to be the only method for determining the relative pathogenicity of strains of *A. scabies*.

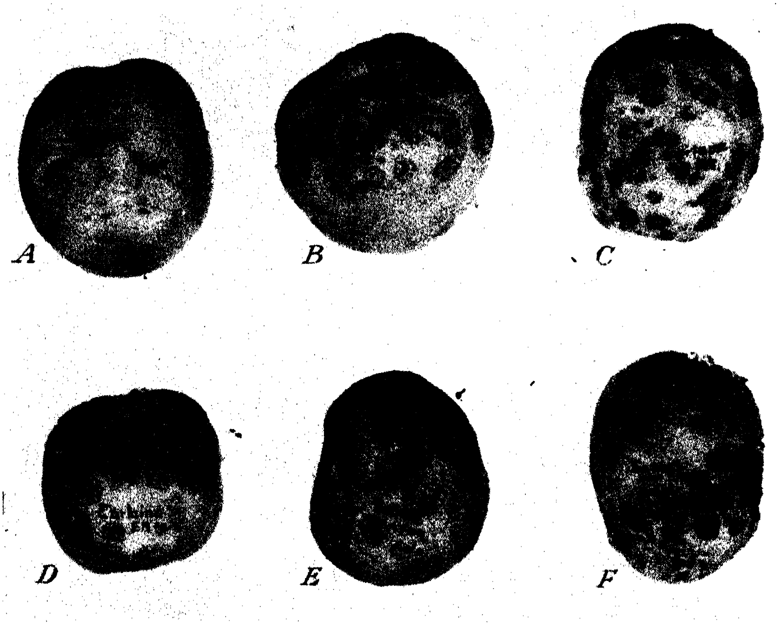


FIGURE 10.—Types of scab pustules on Earlaine tubers produced in scab plots in Rhode Island (*A*); Greeley, Colo. (*B*); Maine (*C*); Minnesota (*D*); Avon, Colo. (*E*); and Michigan (*F*). In Minnesota and Michigan, plots were on peat soil; in the other States on mineral soil. The Avon, Colo., plot was located in an intermountain valley at an altitude of 7,500 feet.

The large number of strains of *Actinomyces scabies* found in soils suggests that variation is occurring in the soil, and that possibly some of these new strains may be more pathogenic than others on the tubers of a given variety of potatoes. Thus a single variety may possibly vary from highly resistant to completely susceptible in the same area, depending on the pathogenicity of the variants present. How rapidly new variants are produced in nature is not known. Some isolates produced very few sectors in cultures; others produced many. Nutrients, temperature, and the hydrogen-ion concentration of the medium did not appear to have much effect on rate of sectoring, which appears to be racial in character.

Most investigators agree that infection by the scab organism is influenced by the reaction of the soil. It is assumed that *Actinomyces* does not infect potato tubers growing in soil at pH 5.0 or below, but that it does infect these growing in soils at pH 6.0 to 7.5. These experiments indicate that certain strains of *Actinomyces scabies* are capable of causing scab on tubers growing in soil with a pH value as low as 5.4.

Field studies showed that some seedling potatoes might be resistant in one section of the country and susceptible in another (figs. 7 to 10), and greenhouse tests indicated that different varieties differed in reaction to the various isolates (table 5).

Although these investigations indicate that scab resistance in potatoes is relatively stable, as is shown by the consistency of pustules on a given variety in a given environment, it is clear that the reaction of a variety may vary with the strain of the pathogen. Much remains to be done on the nature of resistance. Some varieties may have morphological resistance and others physiological resistance. More reliable information on the effect of the various strains of *Actinomyces scabies* on resistance will have to be determined in future studies.

SUMMARY

Single-cell cultures of *Actinomyces scabies* were obtained from potatoes grown on various soil types in different States. They differed in color of mycelium and of pigment produced on modified potato-dextrose agar.

The isolates were unstable and produced variants that were often culturally different from the parents and from each other. One variant studied did not sector during a 3-month period.

No isolate produced cells that averaged more than 2.5 μ long. Some isolates produced spirals, and others did not.

Aerial mycelium was inhibited by high-nitrogen media. Most rapid growth was made on a medium containing thiamine. On such a medium the number of sectors was greater.

On potato-dextrose agar four isolates grew somewhat better at 20° to 24° C. than at 18°. Sectors were produced at all temperatures studied, but those in cultures at 18° were smaller than those at 22° and 24°.

Ten isolates grew on potato-dextrose agar adjusted to pH 5 to 8.5; after 60 days five of these had made very little growth in the medium at pH 5. Sectors were produced at all pH values except 5.

A number of isolates tested on three commercial and five seedling varieties of potatoes differed in pathogenicity. No definite correlation was detected between pathogenicity and cultural or other characteristics. Variants seemed to differ from their parent cultures in pathogenicity. Certain strains produced russeting of normally smooth-skinned varieties.

LITERATURE CITED

(1) AFANASIEV, M. M.
1937. COMPARATIVE PHYSIOLOGY OF ACTINOMYCES IN RELATION TO POTATO SCAB. Nebr. Agr. Expt. Sta. Res. Bul. 92, 63 pp.

(2) BARSS, H. P.
1937. THE VARIABLE PROPERTIES OF POTATO AS A BACTERIOLOGICAL CULTURE MEDIUM. (Phytopath. note) Phytopathology 27: 865.

(3) CLARK, C. F., STEVENSON, F. J., and SCHAAL, L. A.
1938. THE INHERITANCE OF SCAB RESISTANCE IN CERTAIN CROSSES AND SELFED LINES OF POTATOES. Phytopathology 28: 878–890, illus.

(4) DRECHSLER, C.
1919. MORPHOLOGY OF THE GENUS ACTINOMYCES I AND II. Bot. Gaz. 67: 65–83, 147–168, illus.

(5) GOSS, R. W.
1937. THE INFLUENCE OF VARIOUS SOIL FACTORS UPON POTATO SCAB CAUSED BY ACTINOMYCES SCABIES. Nebr. Agr. Expt. Sta. Res. Bul. 93, 40 pp., illus.

(6) KRANTZ, F. A. and EIDE, C. J.
1941. INHERITANCE OF REACTION TO COMMON SCAB IN POTATO. Jour. Agr. Res. 63: 219–231.

(7) LEACH, J. G., DECKER, P., and BECKER, H.
1939. PATHOGENIC RACES OF ACTINOMYCES SCABIES IN RELATION TO SCAB RESISTANCE. Phytopathology 29: 204–209, illus.

(8) LUTMAN, B. F., and CUNNINGHAM, G. C.
1914. POTATO SCAB. Vt. Agr. Expt. Sta. Bul. 184, 64 pp., illus.

(9) ——— LIVINGSTON, R. J., and SCHMIDT, A. M.
1936. SOIL ACTINOMYCES AND POTATO SCAB. Vt. Agr. Expt. Sta. Bul. 401, 32 pp., illus.

(10) MILLARD, W. A., and BURR, S.
1926. A STUDY OF TWENTY-FOUR STRAINS OF ACTINOMYCES AND THEIR RELATION TO TYPES OF COMMON SCAB OF POTATO. Ann. Appl. Biol. 13: 580–644, illus.

(11) OYAMA, T.
1937. VITAMIN B UND DERMATOMYCETEN I. MITT. EINFLUSS DES B-VITAMINS AUF DIE DERMATOMYCETEN. Nagasaki Igakkwai Zasshi 15: 2601–2635.

(12) SCHAAL, L. A.
1940. VARIATION IN THE TOLERANCE OF CERTAIN PHYSIOLOGIC RACES OF ACTINOMYCES SCABIES TO HYDROGEN-ION CONCENTRATION. (Phytopath. note) Phytopathology 30: 699–700, illus.

(13) SHAPOVALOV, M.
1915. EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF THE COMMON POTATO-SCAB ORGANISM. Jour. Agr. Res. 4: 129–134, illus.

(14) STEVENSON, F. J., SCHAAL, L. A., CLARK, C. F., AKELEY, R. V., and cooperators.
1942. POTATO-SCAB GARDENS IN THE UNITED STATES. Phytopathology 32: 965–971.

(15) WAKSMAN, S. A.
1919. STUDIES IN THE METABOLISM OF ACTINOMYCES. II. Jour. Bact. 4: 307–330, illus.

ACQUIRED IMMUNITY FROM CURLY TOP IN TOBACCO AND TOMATO[1]

By James M. Wallace

Formerly *associate pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Since Wingard (*24*)[2] reported that tobacco plants that had recovered from severe stages of the ring spot disease failed to show the usual disease symptoms when reinoculated with the ring spot virus, similar reactions have been observed in several other plant viruses, including curly top (*4, 12, 21*). Price (*15*) has presented an extensive and detailed review of this subject.

The term "recovery" is used in this paper to designate a permanent change in the plants from a severely diseased to a mildly diseased condition without total loss of the causative virus; and the recovery dealt with in these investigations is distinct from a simple masking of symptoms, which results from such factors as environment and natural resistance. With some viruses and under certain conditions, recovery is of such a high degree that it is difficult to distinguish between the recovered plants and the disease-free plants. In other instances the recovered plants continue to show mild symptoms but do not at any time revert permanently to a diseased condition as severe as that before recovery. In all instances where it has been demonstrated that such recovered plants and their vegetative progeny are resistant to injury from reinoculation with the virus of the disease from which they recovered, it also has been shown that the plants continually harbor virus. Although the concentration of virus in the recovered plants may be lower than that in nonrecovered plants, there is obviously some multiplication of virus since it is still present in vegetatively propagated plants many generations removed from the plant in which recovery originally occurred (*13, 14, 21*).

Investigations of recovery from curly top (*21, 22, 23*) have revealed some reactions not previously obtained in similar studies of recovery from other virus diseases of plants. First, it was demonstrated that the acquired immunity from curly top could be transferred from recovered to healthy tobacco (*Nicotiana tabacum* L.) plants by grafting. Later, it was shown that tomato (*Lycopersicon esculentum* Mill.) plants of varieties that commonly have no power of recovery could be "immunized" by grafting with recovered tobacco plants. Further study showed that in plants that had the power of recovery the process of "immunization" took place gradually but was usually completed before the plants showed signs of recovery. These reactions gave strong support to the belief that protective substances were

---

[1] Received for publication April 26, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 213.

involved in this phenomenon. For convenience, therefore, and because of some similarity to immunological reactions in animals, the terms employed in animal immunology are used in this paper. The term "acquired immunity," as used here, denotes an increase in resistance acquired as a result of some specific reaction between the plant and the virus. This acquired immunity consists of an acquired tolerance of the virus harbored by the plants after recovery and also a resistance to injury from reinoculation. The acquired immunity is referred to as "active" in plants that initiate the reaction leading to recovery and in plants propagated vegetatively from such plants. Plants that have acquired immunity by being grafted with recovered plants are designated as "passively immunized."

This paper reports further on the studies of recovery from curly top, especially that phase of the investigations dealing with the transfer of the acquired immunity from tobacco to tomato.

EXPERIMENTAL RESULTS

ACTIVELY ACQUIRED IMMUNITY IN TOMATO PLANTS

The writer has had few opportunities to look for recovery from curly top in commercial plantings of tomatoes in curly top areas, and only limited information has been obtained regarding the frequency of recovery in plants of tomato varieties under natural field conditions. Over a period of 3 years (1939, 1940, and 1941) about 800 curly top infected plants of commercial varieties were under observation in experimental plots, and only 1 plant recovered. In 1939, at Riverside, Calif., a plant of the Riverside variety recovered from a severe stage of disease by producing basal shoots that showed mild curly top symptoms. This plant had been inoculated earlier by means of leafhoppers (*Eutettix tenellus* (Bak.)) from a mixed colony; i. e., leafhoppers from each of several colonies carrying different virus strains had been grouped on a single large beet plant in order to build up a supply for field inoculations. For inoculation, 20 leafhoppers from this composite colony were confined for 7 days to a terminal portion of a branch of each tomato plant by means of a sleevelike celluloid cage. Cuttings were taken from the recovered Riverside plant and grown in the greenhouse for further study. For identification purposes, the vegetative progeny of this plant was designated Clone 2 and, since the original plant recovered and developed a condition of acquired immunity itself, plants of this clone are considered to have had an actively acquired immunity.

When grown in the greenhouse, cuttings of Clone 2 developed into vigorous plants on which it was sometimes impossible to detect curly top symptoms. Cuttings from Clone 2 were slower in forming roots than were healthy cuttings, and, in the early stages, both root and top growths were somewhat retarded. At later stages, growth of potted plants of Clone 2 very nearly equaled that of healthy plants. At times, slight curly top symptoms were discernible but they were never very conspicuous.

Transfers of virus from plants of Clone 2 to healthy plants by means of the leafhopper vector demonstrated that the plants harbored virus that was virulent on tomato. Figure 1 illustrates typical reactions of plants used in such a test for virulence. Shown in this figure are (*A*) a vigorous, almost symptomless plant of the actively immunized clone; (*B*) a healthy plant of the same variety inoculated with virus directly from the immunized plant shown, the transfer being made by leafhoppers; and (*C*) a plant of this same variety after inoculation with a known virulent strain of curly top virus. Both inoculated plants eventually died from the effects of curly top; these reactions were proof that the virus in the immunized plant was as virulent on tomato as was the other known virus strain.

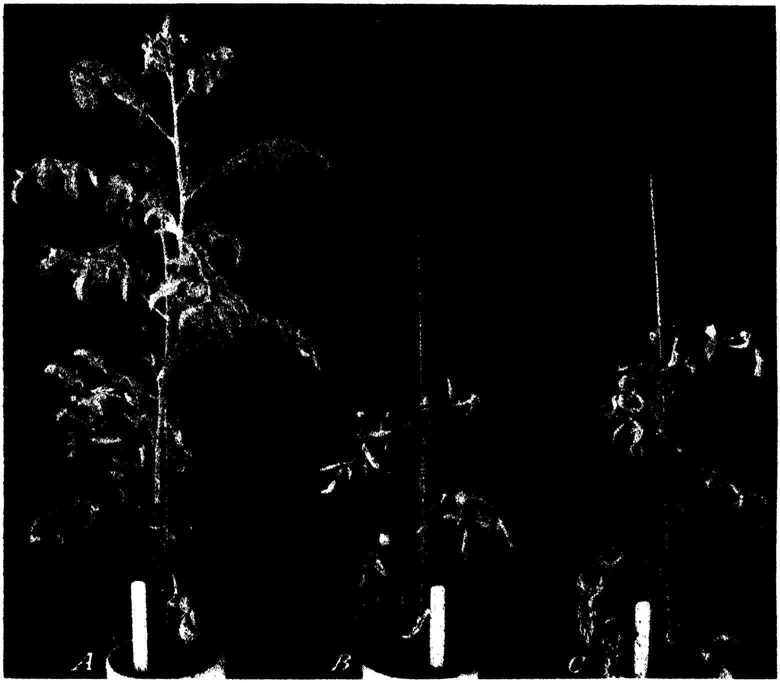


FIGURE 1.—Demonstration of high virulence of virus harbored by plants of actively immunized tomato, Clone 2: *A*, Plant of actively immunized Clone 2; *B*, nonimmunized plant inoculated, by means of beet leafhoppers, with virus from plant *A*; *C*, nonimmunized plant inoculated, by means of beet leafhoppers, with another virus strain known to be virulent on tomato. Both inoculated plants died; this result proved that the virus harbored by Clone 2 was as virulent on tomato as other known virulent strains of the curly top virus.

Clone 2, just described, which had spontaneously recovered from some unknown strain or strains of curly top virus, was tested at different times to two virus strains whose virulence on tomato had been shown. Typical results of such tests are shown in figure 2. Plants *A* and *B* are from immunized Clone 2, and plant *C* is a nonimmunized control. Nonviruliferous leafhoppers were caged on plant *A*, whereas viruliferous leafhoppers carrying a virulent virus strain were caged on plants *B* and *C*. Plant *A*, as expected, was unaffected by exposure to nonviruliferous leafhoppers. A similar cutting from the immunized clone showed no reaction to inoculation by means of viruliferous leafhoppers, but the similarly inoculated nonimmunized control plant developed severe symptoms. Under the conditions of these tests, the plants of Clone 2 were highly resistant to or almost completely protected against further injury from at least two virulent strains of curly top virus.

These studies revealed that recovery, although not common, does sometimes occur in cultivated varieties of tomato and that the reactions involved are apparently identical with those previously observed in the studies of recovery of Turkish tobacco from curly top.

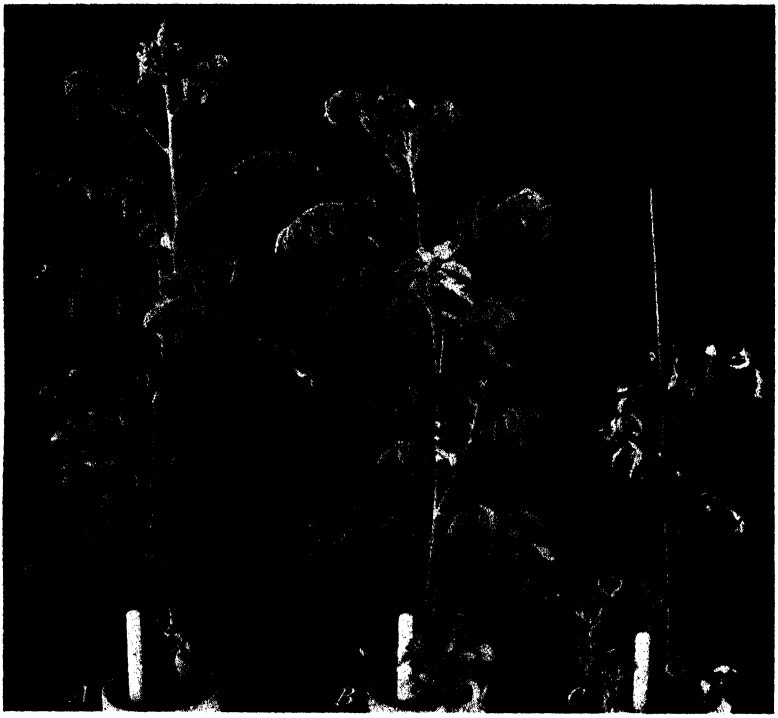


Figure 2.—Effects of reinoculating plants of actively immunized Clone 2 with another strain of curly top virus known to be virulent. *A*, Plant of immunized Clone 2 exposed to nonviruliferous beet leafhoppers. *B*, Plant of immunized Clone 2 exposed to beet leafhoppers carrying a known virulent strain of virus. *C*, Nonimmunized control plant after inoculation similar to that of plant *B*; plant *C* continued to decline and eventually died from the effects of curly top.

PASSIVE IMMUNIZATION OF TOMATO BY MEANS OF TOBACCO PLANTS

Methods

In the first tests made to determine whether the acquired immunity of tobacco could be transferred to tomato plants by grafting, healthy tomato scions were grafted laterally on recovered tobacco plants. Such tomato scions did not develop conspicuous curly top symptoms.

This may have been due in part to the fact that the tomato scions were not in a dominant growth position and thus developed too slowly for normal expression of symptoms. After from 4 to 6 weeks, cuttings were taken from the tomato scions and grown in the greenhouse for further study. Plants grown from such cuttings, or by successive propagations from them, developed almost normally, although they grew somewhat slower than healthy plants and usually showed mild curly top symptoms, indicating the transfer of both virus and protective substances from the tobacco plants to the tomato scions.

In later tests, scions from recovered tobacco plants carrying known individual virus strains were grafted to healthy tomato plants. When this method was followed, some of the tomato plants developed mild symptoms from the beginning, while others developed symptoms typical of the early stages of curly top, sometimes resulting in a severe necrosis on upper portions of the terminal shoot. If the growing point was not killed, new growth developing from the original terminal showed progressively less severe symptoms and, in either case, axillary shoots, sometimes almost free of symptoms, arose from below. On the other hand, tomato plants, infected either by direct leafhopper inoculation or by grafting with tomato plants that had been infected by leafhopper inoculation, developed severe curly top and made no recovery.

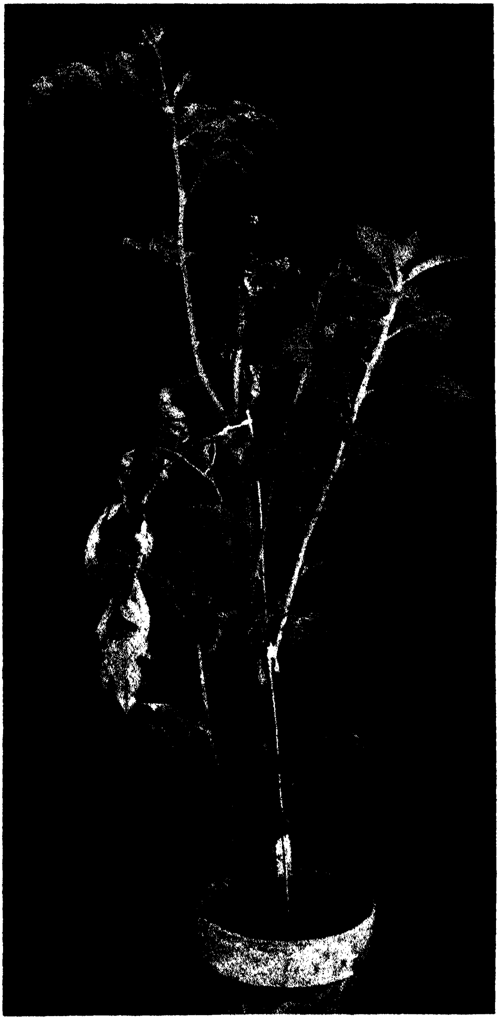


FIGURE 3.—Severe terminal symptoms on tomato plant grafted with a scion from a recovered Turkish tobacco plant carrying curly top virus, strain 8. The main terminal developed severe symptoms and stopped growth, but subsequently almost normal axillary shoots were produced. Photographed 67 days after the graft was made.

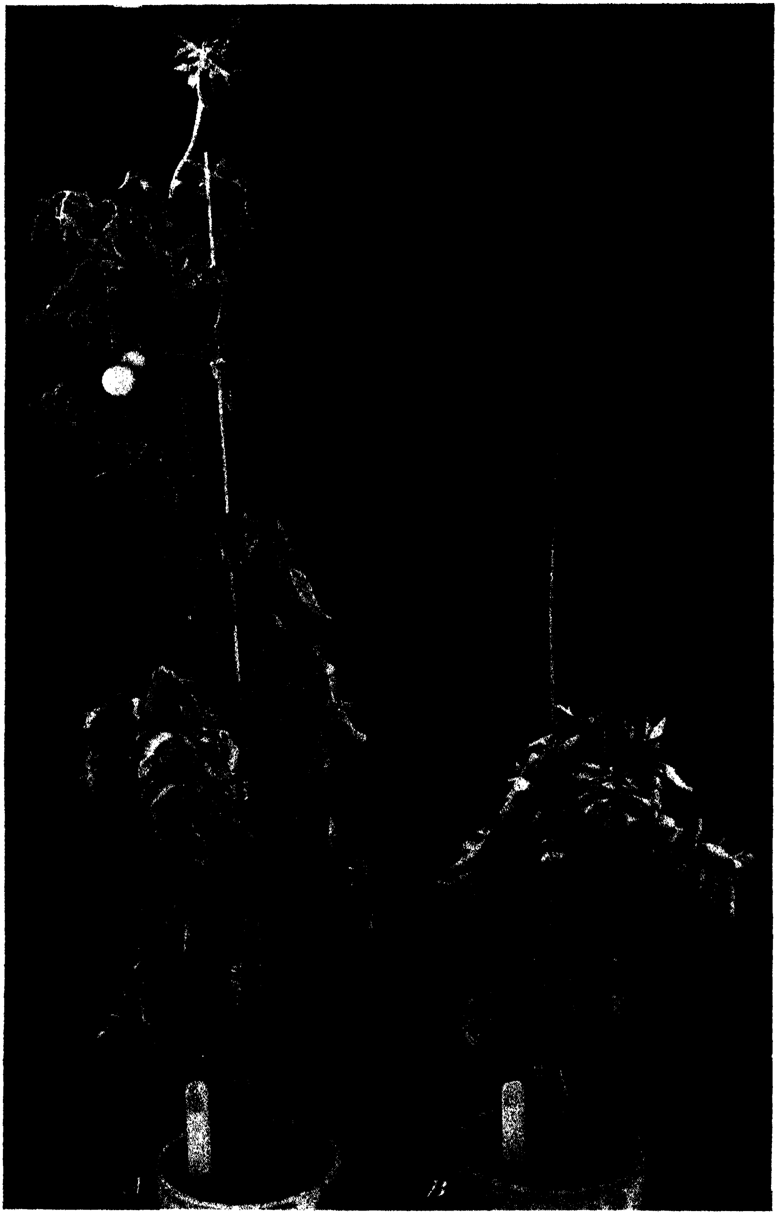


FIGURE 4.—Influence of protective substances on infection of tomato plants with curly top virus, strain 3: *A*, Plant infected by grafting with a tobacco plant that had recovered following inoculation with virus strain 3; *B*, plant infected with the same virus strain by means of beet leafhopper inoculation. Photographed after 64 days.

Figure 3 shows a tomato plant 67 days after it was grafted with a scion from a recovered Turkish tobacco plant carrying curly top virus, strain 8, known to be virulent to tomato. The scion was taken from the terminal of the recovered tobacco plant and was approximately 5 inches in length when the graft was made. The original terminal shoot of the tomato plant was killed, but axillary shoots that were normal in aspect developed from several points along the main stem. Plant *A* of figure 4 was grafted with a scion from a recovered tobacco plant carrying curly top virus, strain 3. Plants of this group did not develop severe symptoms of curly top. For some time the upper parts showed marked leaf rolling, slight yellowing, and some retardation of growth; but, as axillary shoots developed and growth of the original terminal proceeded, the symptoms became less conspicuous. Plant *B* of figure 4 shows the results of infection of a comparable nonimmunized plant with virus strain 3 when the inoculation was made by means of leafhoppers. This photograph was made on the sixty-fourth day after grafting and inoculation of the respective plants. At later stages, plant *B* was dead whereas plant *A* continued growth and was propagated for other tests.

BACK GRAFTS FROM PASSIVELY IMMUNIZED TOMATO TO HEALTHY TOBACCO PLANTS

The tomato plants that were infected by grafting with recovered tobacco could be propagated vegetatively, but cuttings taken from tomato plants infected by leafhopper inoculation always died. When scions from the passively immunized tomato plants were grafted back to healthy tobacco plants, the tobacco developed only mild symptoms, closely resembling those obtained when grafts were made directly from immunized tobacco to healthy tobacco. On the other hand, healthy tobacco plants grafted with scions from diseased, nonimmunized tomato plants developed severe curly top. Figure 5 shows the striking difference in the reactions of Turkish tobacco plants after grafting with scions from the two sources. The scion used on plant *A* came from a diseased, nonimmunized tomato plant. The scion used on plant *B* came from an immunized plant grown from a cutting from a tomato plant previously infected and immunized by grafting with a recovered tobacco plant. Both plants furnishing scions were in a field inoculation test and had received like inoculations. The reactions of the plants shown in figure 5 are representative of the two groups of plants described in table 1, in which the maximum degree of infection is indicated for each plant. These results, which are typical of those repeatedly obtained, show clearly that the tomato plants that had been infected by grafting with recovered tobacco plants could, in turn, confer protection on other healthy tobacco plants, a situation most simply explained by postulating transfer of a protective principle.

If these reactions are to be interpreted as a type of passive immunization, it must be demonstrated that, in these transfers of virus, the virus itself has not been changed in the direction of attenuation. In the course of these experiments, the virus from the immunized tomato plants was tested by using it to inoculate healthy tomato plants with the beet leafhopper as the vector. In all cases of positive transfer of virus, the test plants developed severe curly top. Abundant evidence was obtained by tests of this kind that the virus in the mildly diseased, immunized plants produced severe curly top on healthy plants. This evidence proves that recovery of plants and transfer of acquired immunity by grafting cannot be attributed to lessened virulence of the virus. Of course the hypothesis could be advanced that the virus in the immunized plants exists in an attenuated state but on passage through the leafhopper an increase in virulence takes place. No parallel or analogous instance of such an effect on the virus is known for curly top. The work of Giddings (*6*), involving thousands of inoculations, has given no indication that curly top strains of low virulence are changed in any way on passage through the leafhopper.

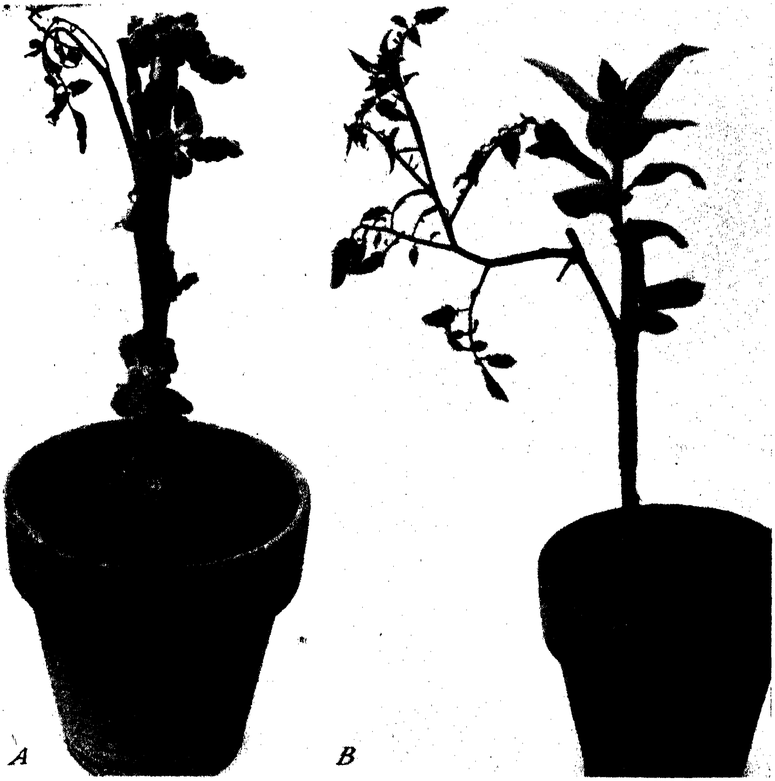


FIGURE 5.—Effects of grafting Turkish tobacco plants with scions from nonimmunized and immunized tomato plants: *A*, Tomato scion from a nonimmunized, leafhopper-inoculated field-grown plant; *B*, tomato scion from a passively immunized plant which had also received a controlled field inoculation. Both field plants furnishing scions had become accidentally infected with tobacco mosaic, but this was not a factor in the reaction of the plants shown.

TABLE 1.—*Reactions of healthy Turkish tobacco plants on which were grafted scions from immunized and nonimmunized tomato plants*

[Greenhouse tests]

Source of scion	Reaction [1] of tobacco stock plant—										Scions alive 40 days after grafts were made
	1	2	3	4	5	6	7	8	9	10	*Number*
Immunized tomato [2]	+	+	[3] 0	+	+	+	+	+	+	+	10
Nonimmunized tomato [4]	+++	++++	[5] 0	+++	++	++++	++++	++++	[5] 0	[5] 0	[6] 1

[1] Indicated as follows: 0=no reaction; +=mild; ++=moderately severe; +++=severe; ++++=extremely severe.

[2] Plant grown from cutting from tomato plant immunized by grafting with an immunized tobacco plant.

[3] Scion lived but made no growth; stock plant not infected, indicating absence of vascular connection between scion and stock.

[4] Tomato plant infected directly by leafhopper inoculation.

[5] Scion died early; apparently no vascular connections formed.

[6] 1 scion remained alive but made no growth; stock plant became diseased.

GREENHOUSE REINOCULATION TESTS ON PASSIVELY IMMUNIZED TOMATO PLANTS

Several different tomato clones that originated from tomato plants passively immunized by grafting with recovered tobacco plants were grown in the greenhouse and tested for resistance to reinfection. In the early tests, plants of these immunized tomato clones were reinoculated on several occasions with unknown mixtures of curly top virus

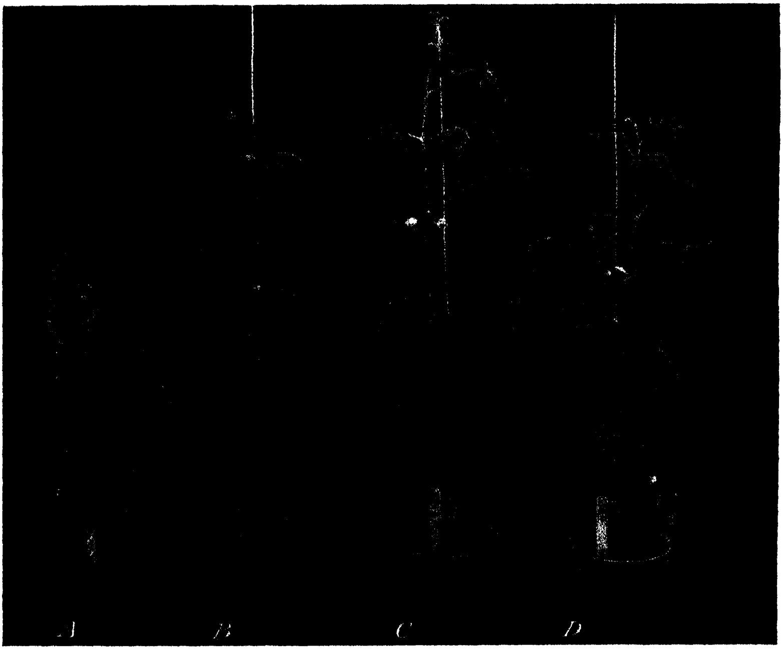


FIGURE 6.—Effects of reinoculation with a virulent strain of curly top virus upon passively immunized tomato plants: *A*, Control plant, showing extremely severe injury; *B* to *D*, plants from passively immunized clones, showing no conspicuous injury from inoculation.

strains. In other tests, two known strains were used individually for reinoculation. Although the virus strains used were virulent on tomato and produced severe symptoms on nonimmunized healthy control plants, the immunized plants showed no noticeable reactions to these reinoculations. The effects of inoculation upon a healthy control plant and three immunized plants, all grown as cuttings, are shown in figure 6. It can be seen that the healthy control was severely injured by the inoculation, but the immunized plants were unaffected. Similar reinoculation tests were made on a sufficient number of plants to justify the conclusion that the tomato plants, after being passively immunized from tobacco, were provided with a high degree of protection against two known strains and certain other unknown strains of curly top virus. Additional data concerning reinoculation of immunized tomato plants are presented later in this paper. In the experiments just described, records were incomplete in regard to the virus strains present in some of the immunized clones. These preliminary data have been presented, however, because they demonstrated passive immunization and because these clones were used in subsequent field tests.

FIELD TESTS OF IMMUNIZED TOMATO PLANTS

Natural Exposure

In 1940 field plantings were made of some of the immunized tomato clones near Twin Falls, Idaho. Actively immunized Clone 2 and four clones of passively immunized tomatoes were included, as well as healthy, nonimmunized plants for controls. One of the passively immunized tomato clones was of the Stone variety and the others were of the Riverside variety. The cuttings were rooted at Riverside, Calif., and shipped to Twin Falls, where they were potted and grown in the greenhouse for about 3 weeks before being transplanted to the field.[3] The controls consisted of cuttings from healthy, nonimmunized Riverside plants; as a further check on the degree of exposure to curly top, seedling plants of both the Riverside and the John Baer variety were included in the field planting.

The plants were set in the field on June 12 in a plot bordered on both sides by sugar-beet plantings. This Idaho district experienced an extremely heavy infestation of beet leafhoppers, and the incidence and severity of curly top in the nonimmunized tomato plants and in the susceptible sugar-beet varieties nearby gave proof that the tomato plants had had a severe curly top exposure. During the early part of the season, before curly top began to appear, the healthy control plants grew much more vigorously than the immunized plants. However, as the season advanced and the disease spread through the controls, the immunized plants continued to grow and increase in size, whereas by September 1 the control plants, numbering 224, were all severely diseased and a large percentage of them had died. Curly top was so severe and developed so rapidly that no edible fruits were produced on the controls. From September 1 until the plants were killed by frost, there was a sharp contrast in the appearance of the immunized lots and the nonimmunized controls. With the exception of the plants of one clone, a high percentage of the immunized plants were in good condition, showing almost normal color, growing fairly vigorously, and producing a light to medium crop of fruit of good quality. Although some fruit ripened on the immunized plants on about the normal date, ripening in general was somewhat delayed and the plants had many green tomatoes when killed by frost. The fruits that matured on the immunized plants were graded as small to medium, and the yield, although good on some plants, was generally lower than would have been expected on plants not affected by curly top.

---

[3] The Idaho field plantings were supervised by Albert M. Murphy, assistant pathologist, Division of Sugar Plant Investigations.

Plate 1 shows a part of the 1940 Idaho plot as it appeared on September 11. The rows of nonimmunized control plants, consisting of severely yellowed or dead plants, are easily located. In contrast, the immunized clones (replicated throughout the plot as single rows of 10 plants each) for the most part show no injury. In the foreground, as marked by the numbered stakes, are shown (1) dead or badly diseased plants grown from cuttings of nonimmunized healthy plants, (2) highly resistant plants of Clone 2 (actively immunized), and (3) highly resistant plants of Clone 3 (passively immunized). Adjacent to and on the right of Clone 3 can be seen a portion of a row of another passively immunized clone. The yellowed plants of this latter group show injury from reinoculation.

TABLE 2. *Reactions of immunized tomato clones and of healthy, nonimmunized controls to curly top exposure in field tests in Idaho*

[Record made October 2, 1940]

Seedling lot or clone No.	Variety and immunization	Virus strains used in immunizing	Plants tested	Plants diseased	Plants showing indicated degree of infection [1]			Plants surviving
					Severe	Moderately severe	Mild	
			Number	*Number*	*Number*	*Number*	*Number*	*Percent*
1	Cuttings, Riverside variety, nonimmunized controls.	None	76	76	76	0	0	26.4
2	Cuttings, Riverside variety, actively immunized.	Mixture [2]	57	[3] 57	7	0	50	100.0
3	Cuttings, Stone variety, passively immunized.	do [4]	39	[3] 39	0	6	30	[5] 92.3
4	Cuttings, Riverside variety, passively immunized.	Strain 9 [6]	61	[3] 61	[7] 6	13	34	86.9
5	Cuttings, Riverside variety, passively immunized.	Mixture [4]	45	[3] 45	0	3	42	100.0
6	Cuttings, Riverside variety, passively immunized.	Strain 9 [6]	28	[3] 28	3	19	6	100.0
7	Seedlings, Riverside variety, nonimmunized controls.	None	74	74	74	0	0	0
8	Seedlings, John Baer variety, nonimmunized controls.	do	74	74	74	0	0	0

[1] All plants in immunized groups not showing symptoms were included as mildly diseased.

[2] Original plant recovered after inoculation with mixture of strains.

[3] All immunized plants carried virus and were therefore considered to be diseased regardless of symptom expression.

[4] Immunization came from tobacco plant that had been inoculated by leafhoppers from stock colony presumably consisting of mixed strains.

[5] 3 plants died from ground squirrel injury; from standpoint of curly top, this group also had 100-percent survival.

[6] Immunization came from tobacco plant that had been inoculated by leafhoppers carrying a single known strain (strain 9) of virus.

[7] This number probably low, 8 dead plants were not included because of uncertainty of cause of death.

The different tomato groups used in this test are listed in table 2, with data on reaction to the curly top exposure and plant survival on October 2. Plants of immunized Clone 6 were known to be low in vigor. Growth was poor, and the plants had shown marked curly top symptoms when grown in the greenhouse without further exposure to infection. Therefore, the poor performance of this clone, as shown in table 2, was very probably not due to reinfection, but rather to the fact that these plants had a lower degree of specific protection against the virus already in them. It is of interest, however, to note that 100 percent of the plants of this clone survived, even though more than three-fourths of them showed symptoms ranging from moderately severe to severe.

The data on survival of plants, as shown in table 2, contribute significant information. From the standpoint of mortality from curly top, 4 of the 5 immunized lots gave 100-percent survival. In Clone 3, shown as 92.3-percent survival, the loss of 3 plants from causes other than curly top was responsible for this group not being recorded as 100 percent. In Clone 4, a total of 8 plants died, but it was not possible to determine definitely whether all of these died from curly top. Even if all of the 8 plants died from curly top, this would make a total loss of only 8 out of 230 immunized plants in the test as a whole. On the other hand, in the controls all of the 148 healthy seedlings were dead on October 2, and only 20 out of a total of 76 plants grown from nonimmunized cuttings were surviving on that date. Of the 20 surviving plants in this latter group, all were severely diseased, and many were surviving only because of the fact that frequent rains and much cloudy, cool weather in September had enabled them to persist.

Some of the plants of the immunized groups, particularly in Clones 2, 3, and 4, developed curly top symptoms decidedly more severe than those shown by the majority of the plants in these groups. The most reasonable explanation of this seemed to be that for the most part the immunized plants were protected to a high degree against injury from the virus strains carried by most of the beet leafhoppers but that some of the vectors transmitted other strains against which the protection by immunizations was not equally effective. It was also evident that some of the immunized lines had a higher degree of protection than others. Other data, presented in a later section, support this explanation of the results obtained.

CONTROLLED INOCULATIONS

At Riverside, Calif., in 1940, field plantings were made of the immunized clones included in the Idaho tests, with the exception of Clone 6. The stock plants of this clone were of such a low degree of vigor that no plants of this line were available. Cuttings from healthy plants and healthy seedlings of the Riverside variety were included as controls. Since natural infestation in this area is usually very light, controlled inoculations can be made in the field, and the desired noninoculated plants, for the most part, remain free of infection throughout the season. These conditions permitted observations on both reinoculated and untreated plants of the immunized groups and on inoculated and noninoculated controls.

Well-established potted plants were transplanted to the field on May 29, and inoculations were made on July 5. Leafhoppers from each of several colonies, carrying different virus strains, were grouped, and 20 leafhoppers from the mixed colony were caged on each plant to be inoculated. All of the curly top strains used were known to be virulent on healthy tomatoes.

The inoculation tests at Riverside gave conclusive evidence that, under the conditions of this experiment, the immunized clones were injured by reinoculation when many virulent curly top virus strains were used. Certainly, one or more of the virus strains used for inoculation were capable of reinfecting the plants and producing severe injury. Another obvious fact was that some of the immunized clones responded differently to this reinoculation. This fact becomes evident upon examination of the data presented in table 3, prepared from records taken on September 16. The tests under natural exposure in Idaho also gave a suggestion of such differences and, of the three passively immunized clones tested in both localities, Clone 4 in both instances showed the most injury from reinfection. A possible explanation for a lower degree of protection in this clone is that the immunization in these plants originally came from a tobacco plant carrying a single strain of virus, whereas in Clones 3 and 5 the immunization came from tobacco plants originally inoculated with a mixture of strains, all of them different from that in Clone 4. As will be brought out later, the degree of protection may vary between immunized clones harboring different strains of the virus.

TABLE 3.—*Reaction of immunized tomato clones and healthy nonimmunized controls to curly top in field inoculation tests at Riverside, Calif.*

[Record made September 16, 1940]

Seedling lot or clone No.	Variety and immunization	Virus strains used in immunizing	Field treatment	Plants tested	Plants showing indicated degree of infection			Plants surviving
					Severe	Moderately severe	Mild	
				Number	*Number*	*Number*	*Number*	*Percent*
1	Cuttings, Riverside variety, nonimmunized controls.	None	Inoculated	20	20	0	0	65.0
			Not inoculated	16	0	0	0	100.0
2	Cuttings, Riverside variety, Clone 2, actively immunized.	Mixture [1]	Inoculated	20	7	10	3	75.0
			Not inoculated	20	1	0	19	100.0
3	Cuttings, Stone variety, passively immunized,	do [1]	Inoculated	19	2	2	15	100.0
			Not inoculated	19	0	1	18	100.0
4	Cuttings, Riverside variety, passively immunized.	Single strain [2]	Inoculated	19	15	2	2	36.8
			Not inoculated	19	0	8	11	100.0
5	Cuttings, Riverside variety, passively immunized.	Mixture [1]	Inoculated	30	11	14	5	93.3
			Not inoculated	30	0	0	30	100.0
7	Seedlings, Riverside variety, nonimmunized controls.	None	Inoculated	10	10	0	0	0
			Not inoculated	8	0	0	0	100.0

[1] Undetermined mixed strains of virus, used in original inoculations of the tomato or tobacco plants in which the immunization was first initiated.

[2] A single known strain (strain 9) of virus, used in inoculation of the tobacco plant in which the immunization originated prior to its passive transfer to tomato.

ROLE OF VIRUS STRAINS IN ACQUIRED IMMUNITY OF TOMATO CLONES

REACTION OF PLANTS OF ACTIVELY IMMUNIZED CLONE 2 TO DIFFERENT VIRUS STRAINS

Inoculation tests of immunized tomato plants under controlled conditions in the greenhouse had proved that the plants were highly resistant to injury from infection with at least two strains of curly top virus that were virulent on nonimmunized plants. The reaction of some of the immunized plants under natural exposure in the field at Twin Falls was interpreted to mean that protection from one strain

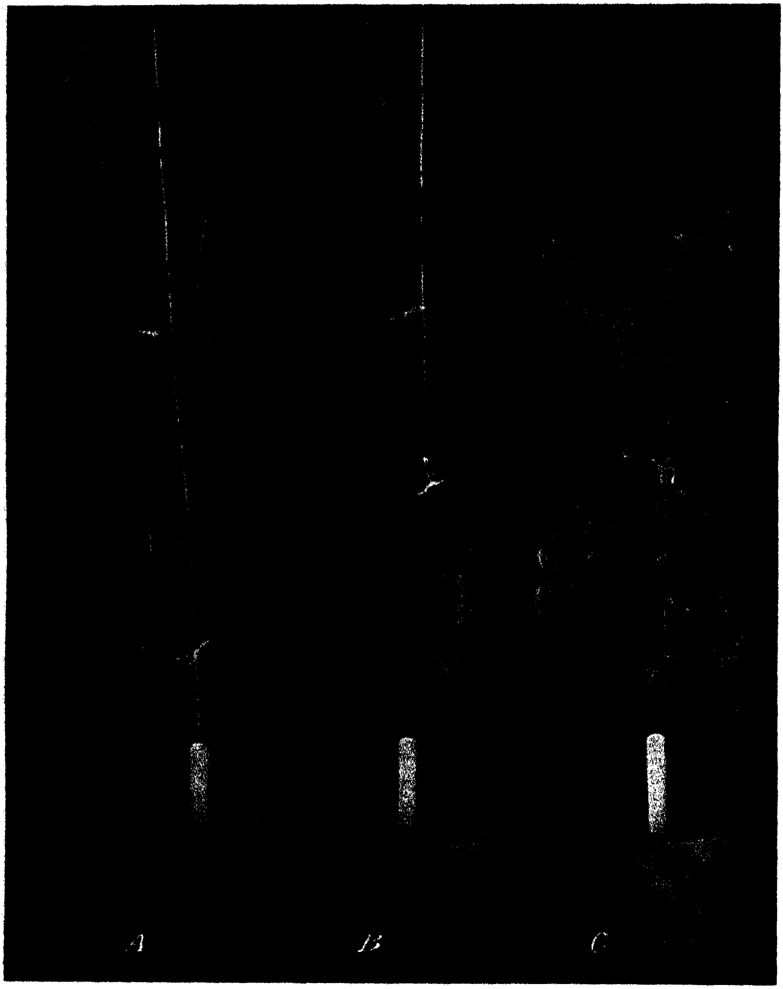


Figure 7.—Representative tomato plants from reinoculation test in which plants of actively immunized Clone 2 were inoculated with different strains of the curly top virus. *A*, Plant inoculated with strain 8; unaffected. *B*, Plant inoculated with strain 66; mild symptoms developed on terminal portions but plant continued growth without being stunted. *C*, Plant inoculated with strain 58; conspicuous symptoms developed; terminal growth weak and chlorotic. Photographed 76 days after inoculation. Of the nonimmunized control plants inoculated at the same time with these three strains of virus, none survived.

of the virus did not give protection against all strains. Artificial-inoculation tests of field-grown plants at Riverside gave conclusive proof that acquired immunity against one strain of the virus was not

effective against all strains, and this phenomenon presented a new phase of the problem for investigation. Although this part of the study is still incomplete, some of the results obtained are reported here.

The previous tests with Clone 2 had demonstrated that the acquired immunity in this clone was effective against two virus strains. This plant material was chosen for further tests against a number of virus strains. These tests demonstrated that virus strains play a significant role in acquired immunity from curly top in tomato. By making tests with a number of strains of curly top virus, it was shown that, depending upon the strain used, the protection existing in Clone 2 ranged from complete to slight. The plants shown in figure 7 are representative of three groups of Clone 2, 76 days after inoculation with three strains of virus respectively. Plant *A*, inoculated with strain 8, was unaffected; plant *B*, inoculated with strain 66,[4] gave a mild reaction; and plant *C*, after reinfection with strain 58, developed severe symptoms.

The three virus strains used in this test are virulent on healthy tomato plants. In the test just described, healthy plants were inoculated in each case and all of them died from curly top before the plants in figure 7 were photographed. Although the plants of this immunized clone reacted quite severely to reinoculation with virus strain 58, the development of disease was much slower than in healthy plants infected with this strain, and usually the reinfected immunized plants survived for long periods. In fact, some of them made a feeble recovery and could be continued by cuttings, but the plants propagated from them were of very low vigor. However, it was evident that the immunized plants were more resistant to virus strain 58 than the nonimmunized plants.

TABLE 4.—*Reaction of plants of actively immunized Clone 2 and of healthy nonimmunized control plants to inoculation with 9 different virus strains*

[The number of plants of each clone tested against each virus strain ranged from 4 to 15]

Tomato clones	Reaction[1] when inoculated with virus strain—								
	1	3	5	6	8	9	58	60	66
Clone 2, immunized[2]	0	0	+++	+++	0	+++	+++	0	+
Clone 1, nonimmunized[3]	++++	++++	++++	++++	++++	++++	++++	++++	++++

[1] Indicated as follows: 0 = no reaction; + = mild; +++ = moderately severe to severe (some plants died after long periods, others made weak recovery); ++++ = extremely severe, causing death of all plants.
[2] Virus strains involved in the initial active immunization were not identified.
[3] Healthy controls.

The reaction of plants of actively immunized Clone 2 to reinoculation with nine different strains of curly top virus has been determined. Table 4 summarizes these data and shows, in comparison, the reaction of healthy nonimmunized tomato plants to the same nine strains. Four strains failed to produce any noticeable effect on Clone 2; the protection, under the conditions of these tests, was complete against those strains. However, the other five strains infected the immunized plants, and four of them caused marked injury. Although this clone was quite severely affected when reinoculated with certain virus strains, the plants were very slow in showing the effects of reinfection; and some of them survived and sometimes made a partial recovery. On the other hand, the healthy controls developed curly top rapidly and all of them died as a result of infection.

---

[4] Strain numbers above 10 are tentative numbers assigned by N. J. Giddings for identification purposes.

Increased manifestation of symptoms following reinoculation of immunized plants seems to be sufficient proof that the virus strain used for reinoculation became established in the plants. Nonviruliferous leafhoppers were fed on some of these plants to acquire virus and then were transferred to differential host plants whereby it could be determined if the second virus strain was present. In this manner, for example, it was shown that virus strain 58 was present in some of the Clone 2 plants that had been inoculated with this strain.

REACTION TO DIFFERENT VIRUS STRAINS OF CLONES PASSIVELY IMMUNIZED AGAINST SINGLE KNOWN VIRUS STRAINS

In order to make a further study of the relation of virus strains to acquired immunity from curly top, tomato plants were passively immunized against 12 individual strains of virus. To accomplish this immunization, Turkish tobacco plants were inoculated separately, by means of leafhoppers, with known curly top virus strains. Forty days after inoculation some of the tobacco plants had made a good recovery, others were in various stages of recovery, and some still showed no signs of recovery. At that time, healthy tobacco plants were grafted with scions from the inoculated tobacco plants, each carrying a different virus strain. The graft-infected plants developed mild symptoms in all instances, proving that the reactions leading to recovery had taken place in the leafhopper-inoculated plants even though some of them had not yet begun recovery. All of these plants produced new growth later, and there appeared to be no consistent differences in vigor and symptom expression between plants infected with different strains of the virus. A similar uniformity was displayed by the tobacco plants that were immunized by grafting with the leafhopper-inoculated plants.

After the acquired immunity against the individual strains of virus was established in the series of tobacco plants, scions from them were grafted to healthy Riverside tomato plants, to serve as the foundation plants from which to establish 12 tomato clones, each specifically immunized against, as well as carrying, a different virus strain. Later, similar graft transfers were made from immunized tobacco to plants of the tomato varieties John Baer, First Early, and Break o' Day. In some of these experiments, comparable tomato plants were grafted separately with scions from an immunized tobacco plant and with scions from a tomato plant that had been immunized previously from this same tobacco plant. This was done to determine whether the degree of protection conferred on tomatoes was affected by the source of protective substances. From the data so far obtained, there was no good evidence that such was the case. For instance, with a given strain of virus, tomato plants usually reacted about the same when infected by grafting directly with an immunized tobacco plant as when the infection came from a tomato plant which had previously been passively immunized from that same tobacco plant. Some differences in reaction appeared but they were not consistent.

It has been mentioned already (p. 202) that Turkish tobacco plants immunized against the individual virus strains were quite uniform in appearance and vigor. Thus it appeared that tobacco plants recovered to approximately the same degree regardless of which strain of virus was involved. However, when tomato plants were passively immunized against different strains of curly top virus, the results indicated that the degree of immunization attained by the plants varied widely with different virus strains. This was shown by variations in both the initial reaction of the tomato plants after they were grafted with immunized tobacco scions and in the vigor of the clonal propagations from these tomato plants. Tomato plants grafted with

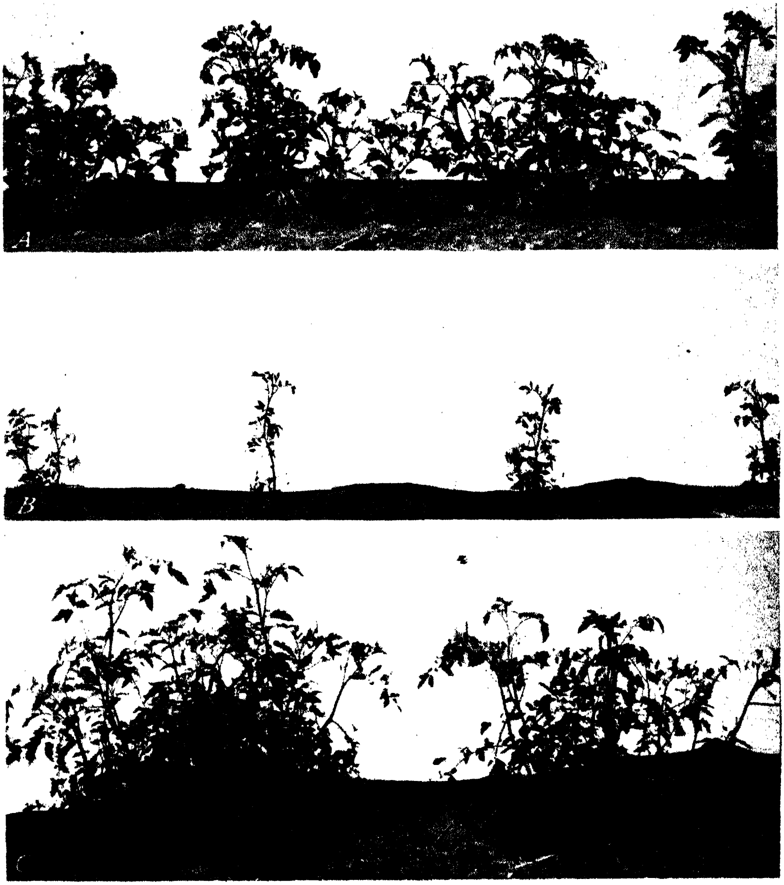


FIGURE 8.—Reduction in vigor of immunized tomato clones, and variation between clones immunized by different strains of curly top virus: *A*, Individuals of clone immunized by virus strain 3; *B*, individuals of clone immunized by virus strain 55; *C*, healthy clonal plants for comparison. Cuttings were made at same time. Photographed 2 months after transplanting to field. Healthy control plants grown in 3-foot spacings, others in 2-foot spacings.

immunized tobacco scions carrying certain virus strains developed severe terminal symptoms and then produced mildly diseased axillary shoots (fig. 3). When certain other virus strains were used, the graft-infected tomato plants showed no severe initial shock such as that previously described (fig. 4). With still other virus strains, infection of tomato plants by graft transfer from immunized tobacco produced a severe reaction from which the tomato plants either failed to recover or made only a feeble recovery. Cuttings from the immunized tomato plants that were not severely affected after grafting with immunized tobacco, and cuttings from tomato plants that made a strong recovery after a severe initial reaction, grew vigorously and showed very mild curly top. Clones of the tomato plants that acquired a low degree of protection against some of the virus strains were low in vigor and showed marked injury from curly top.

Variations in vigor and symptom expression among the various immunized tomato clones, each carrying a different strain of the virus, show up strikingly prior to any reinoculation. Figure 8 shows plants of a healthy clone and of two clones immunized by different strains of curly top virus, grown in the field and not reinoculated. The plants immunized by strain 3 were of normal color and showed no curly top symptom except a slightly retarded growth. Plants of the clone immunized by strain 55 were slightly chlorotic and grew very slowly, but flowered and produced a few small fruits late in the season. On the basis of vigor and symptom expression, the 12 immunized clones used in these studies ranged from very sickly, slow-growing plants to plants that were normal except in rate of growth. In some instances plants of a particular clone, although small to medium, showed none of the usual curly top symptoms. Plants within a given clone were quite uniform in growth and general appearance.

Inasmuch as the tobacco plants in which the immunizing process was initiated and the tomato plants on which the protection was conferred were not all of single genotypes, some of the variability may have come from this source. However, because of the very uniform reaction in tobacco and general concordance among tests with various tomato stocks, effects of variability of the host plants are probably not the significant factor. From the evidence so far obtained, it seems that the immunization acquired by tomato is chiefly influenced by the virus strain itself and the reactions that it sets up.

Although field tests under natural exposure yield much worth-while information, controlled cross-inoculation tests are necessary to determine the relation of virus strains to acquired immunity from curly top in tomatoes. This represents a protracted study, which is still in progress; but some of the preliminary data can be presented.

Several tomato clones, each passively immunized by grafting with tobacco plants that had recovered after infection with single strains of the virus, were subjected to reinfection tests with various strains of the virus. The results of these tests, so far as they have gone, are summarized in table 5. In most instances, from 10 to 20 plants of each clone were inoculated with each virus strain used. The data show that, under the conditions of these tests, plants immunized by one strain may be protected against many strains but that the protection given by one strain of virus does not correspond with that given by some other strain. Of especial interest are the reactions of the clones immunized by different virus strains to reinoculation with strains 9 and 75. The intensity of curly top reactions on some of the immunized clones in these tests might, at first glance, indicate a greater virulence in these two strains; nevertheless all the other virus strains likewise had a lethal effect on the controls. It is to be noted that the tomato plants immunized by strain 55 were completely protected against both 9 and 75, but that strains 5 and 6 both injured to some degree plants immunized by strain 55. In view of the fact that other data presented in this paper have established good evidence that protective substances are involved in recovery and acquired immunity from curly top, the strain specificity just described is interpreted as further evidence that the protective substances are of an immunologic nature. The failure of related virus strains to provide cross-immunity has been demonstrated on several occasions in the field of animal pathology (*11*, *16*).

TABLE 5.—*Reactions of immunized tomato clones carrying single virus strains to inoculation with various strains of curly top virus*

[From 10 to 20 plants of each clone were tested against each virus strain]

Plants immunized against virus strain No.	Reaction [1] when inoculated with strain—									
	3	5	6	8	9	53	55	56	60	75
3	0	++	++	0	+++	0		0		0
8	0			0	+++	0	0			0
53	0	0	0	0	++	0	0		0	++
55	0	+	+	0	0	0	0			0
56	0			0	++	0	0	0	0	+++
Controls (nonimmunized)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

[1] Indicated as follows: 0=no reaction; +=mild; ++=moderately severe; +++=severe; ++++=extremely severe, causing death of all plants.

DISCUSSION

In contrast to other virus diseases of plants that have been studied in relation to recovery and acquired immunity, curly top virus is restricted to the phloem and does not normally invade parenchyma tissues (*2*). Inoculation, except with the vector, gives a very low percentage of infection, apparently because the virus must be introduced directly into the phloem cells. Bennett (*3*) has shown that the virus may invade healthy mature leaves of sugar beet and multiply in them. There are, however, no primary localized symptoms at the site of inoculation such as commonly occur with the ring spot group of viruses. Instead, the symptoms of curly top appear after systemic invasion of tissues that have not passed a certain stage of maturity. Because the tissue relationship of curly top virus and the type of symptoms and manner in which they develop differ so distinctly from other viruses in which recovery has been studied, it was of particular interest to discover this recovery reaction in curly top affected plants.

Several workers have expressed the opinion that, in view of the lack of information on the nature of the reactions involved in recovery of plants from virus diseases and the accompanying protection, it is preferable to refer to this condition of the recovered plants as "acquired tolerance." Valleau (*19*, *20*) has presented strong arguments against the use of the terms "recovery" and "acquired immunity" in connection with the reactions of tobacco plants affected with ring spot. Regarding the mechanism involved in these phenomena, Price (*14*) and Valleau (*19*) seemed to agree that patterned leaves are produced as long as the growing point has not been invaded and that, once the embryonic cells become invaded, patternless leaves are formed. Price (*14*) offered the hypothesis that ring spot virus reaches its maximum concentration in and exerts its maximum effect upon only those cells that are nearly mature at the time they are infected; and, further, invasion of embryonic cells is not accompanied by maximum increase in virus nor by severe injury to the cells. Thus embryonic cells, once invaded, become adapted to the presence of the virus and establish an equilibrium with it. Price recognized that, regardless of the correctness of this hypothesis, it still did not reveal the fundamental nature of the mechanism involved; he expressed the opinion that these reactions are sufficiently similar to many known immune reactions in the field of animal viruses to be accepted as examples of acquired immunity.

Valleau (*19, 20*) contended that, in the case of ring spot and certain similar viruses, the reactions involved in recovery are in no way immunological. He agreed with Price that the acquired tolerance is cellular, but suggested that embryonic cells become tolerant of the virus because of the presence of less virus-precursor materials or because of a less rapid change of such materials into virus. Other workers (*1, 8, 10, 18*) have suggested a somewhat similar explanation for instances of cross-protection, wherein a second virus fails to produce symptoms when inoculated into a plant previously invaded by a closely related virus. It seems logical to assume that certain cell constituents are essential for virus multiplication, and if such is the case the essential materials in a cell invaded by a given virus may become exhausted or at least be used as fast as they are synthesized. Under such conditions it would hardly be expected that a second virus, requiring the same materials as the virus already present, would become established in concentrations sufficient to produce its symptoms when inoculated into cells that are, as stated by Valleau (*19*), "already parasitized to their limit."

Price (*13*) grafted from ring spot recovered plants to healthy plants and found that typical symptoms of the severe stage always developed on the graft-infected plants, the reaction being identical with that on plants infected by the leaf-rubbing method. He stated that, if protective substances were present in the recovered plants, they were not detected.

In the case of curly top, the discovery that usually tobacco plants developed only mild disease symptoms after being grafted with recovered tobacco plants strongly indicated that protective substances were present in recovered plants (*23*). This conclusion was supported by the fact that the recovered plants contained virus that caused severe curly top when transferred by the leafhopper vector to healthy plants. When reinoculation tests had shown that recovered tobacco plants and their vegetative progeny were unaffected by reinoculation with curly top virus and, further, that tobacco plants that were infected by grafting with recovered plants were provided with a similar protection (*23*), it was concluded that in this instance the recovery reactions were of an immunologic nature. Plants that recovered from a severe stage of curly top and were unaffected by reinoculation were described as having an actively acquired immunity. The failure of a healthy tobacco plant to develop severe curly top when grafted to an immunized plant was attributed to a type of passive immunization. Because the condition of immunity acquired by plants that recovered from curly top could be transferred through grafts to other plants, it has been possible to carry these investigations much further than in other instances of recovery of plants from viruses, such as tobacco ring spot, where recovered plants confer no protection on plants to which they are grafted.

Wallace (*23*) has shown that, under conditions about optimum for rapid curly top development, tobacco plants inoculated by means of leafhoppers required a period of about 20 days before they reached a condition that permitted them to confer maximum protection on healthy plants by passive transfer. On the other hand, if scions taken from leafhopper-inoculated plants on the fifth day following inoculation, prior to any showing of curly top symptoms, are grafted on healthy tobacco plants, severe symptoms are shown by the stock plants. It was therefore concluded that passive transfer of protection from plant to plant by grafting resulted from some reaction or change that occurs in leafhopper-inoculated tobacco plants sometime between the fifth and twentieth day after infection. Graft transfers at intermediate periods gave results that indicated that the reaction had begun within 10 days but was not completed on the fifteenth day after the plants were infected. Each of the time periods mentioned above actually may be longer by about 7 days, since in tobacco phloem connection between scion and stock usually requires 7 days or more. Thus, there is the period from time of grafting to time of phloem union, in which the reaction may continue in the scions before phloem materials can move into the stock plants. At the time the grafts were made, the 5-day infected plants showed no curly top symptoms, but the 20-day infected plants showed severe symptoms. On the basis of other tests with the curly top virus, it is believed that, if there is a difference in virus concentration between plants infected 5 days and plants infected 20 days, the scions from the 20-day infected plants contain the higher concentration. At any rate, the virus concentration in the respective scions is not believed to have been responsible for the results obtained in these tests. Plants infected by grafting, regardless of the source of scions, certainly must receive more virus than can be introduced by a small number of leafhoppers, yet infection from leafhopper inoculation usually causes severe symptoms. Leafhopper-inoculated tobacco plants and plants grafted with scions from short-time-inoculated plants sometimes develop only mild symptoms when the incubation period is long. In graft infections a delay of a few days after the graft union is formed before virus moves into the stock may permit the completion of the protective reaction before the terminal of the stock is invaded.

The evidence that tobacco plants develop some type of defense against the virus, enabling them to recover, became more striking when it was shown that, by grafting with immunized tobacco plants, it was possible to immunize tomato varieties that rarely recover and acquire immunity actively when inoculated by means of leafhoppers. The effectiveness of this method also demonstrated conclusively that, whatever the defense mechanism is, it can be transferred through grafts to other plants and can function there in providing protection. After tomato plants have been passively immunized from tobacco plants, this condition can be passed on from these plants to other tomato plants by grafting. If, on the other hand, healthy tomato plants are grafted with diseased nonimmunized tomato plants they become severely affected with curly top and do not recover. Such results cannot be explained on the basis of invasion of embryonic tissues, since the opportunity for invasion of the graft-infected plants is the same in either case. The different response of tomato plants infected from immunized and from nonimmunized scions must, therefore, be caused by a difference in the scions themselves.

That invasion of immature tissues may play some role in acquired immunity from curly top is indicated by some of the results obtained in the studies with tobacco. Wallace (*21*) reported that, when tobacco plants were topped at the time they were grafted with an immunized scion, the symptoms that developed on the first axillary shoots were usually fairly severe for a time. However, such shoots recovered sooner than similar shoots on plants infected by leafhopper inoculation or by grafting with nonimmunized scions. Although this reaction might indicate some relation between stage of tissue differentiation and invasion by the curly top virus, such a relation would be the opposite of that existing in the case of invasion by the ring spot virus; invasion in the earlier stages by curly top causing a severe reaction, whereas a similar invasion by ring spot virus produces symptomless tissues.

Tobacco plants that are not topped after grafting with an immunized scion usually develop mild symptoms from the beginning. Occasionally, however, an untopped plant may develop quite marked symptoms; this is particularly true if the graft-infected plant is closely approaching or has reached the flowering stage. Plants that have shown very mild symptoms for a long time sometimes develop conspicuous symptoms if the plants are topped so as to force new growth from axillary buds. These reactions, and also the increased symptoms on new growth of plants that are topped at the time they are grafted with an immunized scion, are believed to result from an increase in virus concentration through the movement of virus, and possibly of materials used in virus multiplication, into these areas from other parts of the plants. It is known that viruses move rapidly in the phloem toward regions of food utilization or storage. If this rapid movement results from mass flow of the liquid contents of the phloem, as has been suggested by Bennett (*3*), it would be expected that viruses would become highly concentrated in areas receiving large quantities of food materials from other parts of the plant. Bennett and Esau (*4*) found that the concentration of curly top virus increased rapidly in seeds of sugar beet as the seeds developed on curly top affected plants. In consideration of the above points, it would seem that in the plants immunized from curly top the mild symptoms and lessened injury are due to the fact that the virus concentration usually is held at a low level, i. e., at concentrations too low to produce normal injurious effects. The nature of the mechanism that causes this condition is not understood. The studies reported here indicate very strongly that plants recover and acquire this type of protection from curly top as a result of some reaction between the virus and certain materials in the plants, resulting in the production of specific substances that either have an inhibiting effect on the virus directly or else bring about some change in the plants themselves, enabling them to tolerate the virus without becoming seriously injured.

The role of curly top virus strains in acquired immunity is particularly interesting. Turkish tobacco plants seem to recover to about the same degree regardless of the strain of virus in them. However, when graft transfers are made from tobacco to tomato plants, a high degree of protection is acquired by tomato against some of the virus strains and protection of lower degree is acquired against other strains. The conclusion that this difference exists is based on the observation that immunized tomato clones, carrying different strains of virus, vary widely in vigor and severity of symptoms. There has been some indication that individual plant variation has some influence on the degree of protection acquired by tomato. Slight genetic differences in the tobacco plants that develop active immunity or in the tomato plants that are passively immunized could be responsible for some of the variations in the degree of immunization acquired and maintained. Incomplete studies along this line indicate that if a sufficient number of seedling tomato plants are passively immunized against a virus strain that consistently provides a low degree of protection in tomato, an occasional tomato plant in the group may acquire protection of a much higher order, from which a vigorous clone can be established. However, the present belief is that the virus strain itself and the reactions it sets up in the plant account for most of the observed variation between tomato clones immunized by different strains of curly top virus.

Another interesting reaction is revealed by the discovery that immunized tomato plants carrying a certain virus strain or combinations of strains are unaffected by reinoculation with some strains and are injured quite severely by others. As in other phases of this study, the question arises as to whether these data provide any evidence concerning the nature of recovery and acquired immunity from curly top. In attempting to answer this question, a search for analogous reactions in the field of animal viruses has been made. According to Rivers (*16, p. 215*), there are three distinct types of the virus of foot-and-mouth disease, which, although they cannot be distinguished from one another clinically, do not produce cross-immunization. Rivers points out also that there are two types of the virus of vesicular stomatitis of horses, producing identical clinical pictures, but that animals recovered from one are not immune from the action of the other. Another example can be cited in the case of the influenza virus. There are different types of this virus and, further, there are different strains of a given type. Some of the strains of human influenza A virus have been shown to differ widely in antigenic make-up (*11*). This fact has been demonstrated both by reciprocal cross-neutralization tests and cross-immunity tests. Thus, it seems that a plurality of types exists in certain animal viruses whereby a particular virus may consist of two or more strains that differ in their immunologic reactions. In tomato plants, the failure of immunity acquired against one strain of the curly top virus to be effective against all strains of the virus is similar in some respects to the animal virus reactions just described.

Putting aside connotations of terminology involved in the word "immune," the objections most commonly expressed to the findings that plants may acquire immunity against a virus disease are based on the fact that the plants do not completely recover from the disease symptoms and that the causative virus is at all times present in the plants. In other words, insistence is made that recovery or acquired immunity be limited to return to a virus-free condition. These objections are based in part on the assumption that in the field of animal immunology no analogous conditions exist and that animals that recover and acquire immunity from a virus make a complete recovery from disease symptoms and become free of the virus. Good evidence has already been obtained that immune animals do not always become virus-free, and some suggest that they may never reach this condition. Rivers (*16, 17*) cites instances where the viruses of certain diseases have been demonstrated to be present in recovered animals for quite a long period after the symptoms of the disease had disappeared. In fact, it has been suggested that the lasting immunity from many diseases of man and lower animals may actually be dependent upon the persistence of virus (*16, 17, 25, 26*).

There is such a wide difference between plant and animal organisms that it could hardly be expected that they would present identical pictures after acquiring immunity against infectious agents. Higher plants are in a continuous process of growth, particularly when kept in a vegetative state. New tissues are being formed at a rapid rate, perhaps much faster and more generally than in animals, and these tissues very probably produce a continuous supply of materials needed for virus multiplication. The formation of new tissues likewise furnishes susceptible tissues in need of protection. A small cutting from a plant that is immunized against a virus disease increases in size manyfold. Under such conditions, would it not be expected that plants would require a far higher degree of immunization if they are to be protected to the same extent as immunized animals?

The argument is frequently put forth that the wide difference between plant and animal circulatory systems makes it doubtful whether plants can develop a defensive mechanism similar to the antibody formation of animals. In the case of curly top, the virus is restricted to the phloem and thus exists in a uniform medium. If immune reactions occur in the plant virus field, it seems somewhat more probable that they would be found in phloem-restricted viruses. If protective substances are produced in the case of a phloem-restricted virus they would have to exist and operate only in one type of tissue in order to provide a defense against the virus. On the other hand, with viruses that are not restricted to the phloem, the defense mechanism would be required to operate in all of the different tissues invaded by the virus. Under such conditions, it would seem less likely that effective protection would be attained.

If plants that recover from curly top actually contain some type of protective materials, the failure of the leafhoppers to transmit them, assuming mere quantitative considerations are not involved, suggests that these materials are either inactivated in the leafhopper or that they are screened out in some manner so that they do not reach the salivary glands of the insect. The leafhopper apparently separates the virus from the protective substances or else acquires and transmits only free virus, i. e., virus that has not been acted upon by protective substances.

Recovery and acquired immunity from curly top cannot be explained satisfactorily by curtailment or exhaustion of essential materials for virus reproduction or by invasion of embryonic cells. In the first place, in sugar beets, which do not recover and acquire immunity from curly top, ordinary cross-protection between virus strains does not occur. Carsner (*5*), in his early studies of curly top, showed that beets affected with a mild form of the disease were neither immune from nor more resistant to severe forms of the disease. Giddings (*7*) has recently shown that previous invasion of sugar-beet plants by less virulent strains of virus does not protect against other strains of high virulence superimposed upon the first and, further, that strains of high virulence do not prevent strains of low virulence from becoming established. It has not been possible to study the matter of simple strain protection in tomatoes, because all of the known strains of the curly top virus either fail to infect or else are extremely virulent on this host. Yet, after tomato plants become immunized, a certain degree of cross-protection between virus strains becomes effective. This type of cross-protection in immunized plants results from a specific reaction in the plant in which the immunity originates. If it were simply a mildly reacting strain of virus protecting against a related, more virulent strain, then the immunity against one strain of curly top virus would be expected to protect the plants against all others, because the strains of this virus are certainly very closely related. Furthermore, the mildly diseased condition of the immunized plants and their resistance to reinfection can hardly be explained on the basis of the presence of a mildly reacting strain of virus, since, in the experimental work here reported, the tomato plants were immunized against strains of high virulence that persisted in the plants without change.

The demonstration that a period of time is required for tobacco plants inoculated by means of leafhoppers to reach a condition in which they can confer passive protection on other plants is evidence that the condition of acquired immunity involves the production of protective substances within the plant as a result of its being infected with the virus.

The fact that tomato plants of cultivated varieties, which rarely initiate the reaction leading to recovery and acquired immunity, can be provided with this condition when they are grafted with immunized tobacco plants is difficult to explain other than on an immunologic basis. The condition eventually reached in such plants suggests a type of passive immunization which partly protects the plants during the early stages of disease and thereafter incites an active production of protective substances by the recipient plant. Such a reaction differs from the usual conception of passive immunization in the field of animal pathology, but Kolmer and Tuft (*9, p. 34*) suggest a similar phenomenon in the following statement: "Acquired immunity occurs in two distinct forms: (1) *active* and (2) *passive*. A mixed form may exist, brought about by a combination of factors necessary for the development of the other two."

SUMMARY AND CONCLUSIONS

Tomato plants of cultivated varieties become severely diseased when infected with the curly top virus either by leafhopper inoculation or by grafting with other tomato plants that have been infected by means of leafhopper inoculation. If, on the other hand, they are grafted with Turkish tobacco plants in which recovery, or the reaction leading to recovery, has occurred, the tomato plants acquire an immunity similar to that observed in recovered tobacco plants. Since the tomato plants rarely show any tendency to initiate the recovery reaction, this transfer of immunity may be considered a type of passive immunization in plants, although it differs from ordinary passive immunization in animals. Actually, this immunization may be passive only in the sense that certain substances are transferred from the immunized tobacco plants that protect the tomato plants to some extent during the early stages of disease and incite the production of like protective substances in the recipient plants. The retention of the acquired immunity in the tomato plants through many successive propagations, after it once becomes established, suggests that an active process is in operation.

By the use of Turkish tobacco, which always recovers from curly top, it was possible by graft transfer to immunize tomato plants against individual strains of the curly top virus. Immunized tomato plants varied widely in vigor and symptom manifestation according to the virus strain used. Clonal plants propagated from the immunized plants also showed the same wide variation of response, ranging from vigorous, at times symptomless, plants to those of low vigor with conspicuous curly top symptoms. These variations among tomato clones immunized against different virus strains were fairly consistent, and for the most part seemed attributable to the virus strains rather than to individual plant variation.

Tomato clones immunized by single virus strains, when tested by reinoculations which superimposed different virus strains, showed high protection against some strains and less against others. These reactions indicated a definite specificity of immunization. The protection given by one virus strain might be equally effective against many strains, but occasionally the protection was found to be relatively low both from the standpoint of numbers of strains and degree of injury caused by individual strains. These results were confirmed by performance of clones of immunized tomato plants grown in the field under heavy natural exposure to curly top.

Since there are no known strains of curly top virus that react mildly on tomato, it has not been possible to determine whether a mildly reacting strain in a nonimmunized tomato plant would protect against the more virulent strains. However, in tomato plants immunized against and carrying virulent strains of the virus, cross-protection of a kind has been demonstrated. This differs from ordinary cross-protection in that (1) the mild symptoms of the immunized plants are not due to the presence of a virus strain of low virulence and (2) the degree of protection varies from complete to slight, depending on which strain of virus is carried by the immunized plants or which strain is used for reinoculation of the immunized plants. Since all of the virus strains used in these studies are virulent on tomato and since the virus strains have remained stable over a long period of testing on numerous differential plant hosts, it seems most certain that the recovery and acquired immunity from curly top cannot be explained by strain antagonism.

Experiments dealing with graft transfer from immunized plants to healthy plants, in comparison with similar transfer from nonimmunized to healthy plants, led to the conclusion that recovery and acquired immunity from curly top are not correlated with virus invasion of immature tissues.

Recovery and acquired immunity from curly top in tobacco and tomato seem to differ fundamentally from similar phenomena described for other plant viruses and seem closely comparable to reactions known for animal viruses. The experiments that deal with passive transfer of protective substances and the time element involved in the recovery process, during which the protective substances are generated, support this position. Furthermore, the specificity among virus strains, with respect to their immunizing potentialities and the degree of protection conferred either actively or passively, corresponds with results reported for animal virus strains. The persistence of virus in the recovered plants no longer excludes these plant reactions from the field of immunology, as more evidence is obtained that immunity from certain animal viruses may actually depend on the continued presence of the causative virus.

This study on plant reactions to curly top virus has given the following findings: Regularly occurring recovery in tobacco; acquired resistance of recovered plants to injury from reinoculation; persistence in recovered plants of curly top virus not lessened in virulence; evidence of a time factor involved in the reactions leading to recovery; evidence that this recovery and acquired resistance does not result from invasion of embryonic tissues; proof of transfer by grafting of the acquired condition of tolerance and resistance from a recovered plant, not only as an intraspecific transfer (tobacco to tobacco) but as an interspecific passage (tobacco to varieties of tomato that very rarely initiate the recovery reaction); and, finally, evidence of the striking specificity exhibited by different strains of the virus. This whole range of experimental evidence clearly indicates that the phenomena are immunologic in nature.

LITERATURE CITED

(1) Bawden, F. C.
1934. STUDIES ON A VIRUS CAUSING FOLIAR NECROSIS OF THE POTATO. Roy. Soc. London, Proc., Ser. B, 116: 375–395, illus.

(2) Bennett, C. W.
1934. PLANT-TISSUE RELATIONS OF THE SUGAR-BEET CURLY-TOP VIRUS. Jour. Agr. Res. 48: 665–701, illus.

(3) ———
1937. CORRELATION BETWEEN MOVEMENT OF THE CURLY TOP VIRUS AND TRANSLOCATION OF FOOD IN TOBACCO AND SUGAR BEET. Jour. Agr. Res. 54: 479–502, illus.

(4) ——— and Esau, K.
1936. FURTHER STUDIES ON THE RELATION OF THE CURLY TOP VIRUS TO PLANT TISSUES. Jour. Agr. Res. 53: 595–620, illus.

(5) Carsner, E.
1925. ATTENUATION OF THE VIRUS OF SUGAR BEET CURLY-TOP. Phytopathology 15: [745]–757, illus.

(6) Giddings, N. J.
1938. STUDIES OF SELECTED STRAINS OF CURLY TOP VIRUS. Jour. Agr. Res. 56: 883–894, illus.

(7) GIDDINGS, N. J.
1940. CURLY-TOP VIRUS STRAINS. (Abstract) Phytopathology 30: 786.
(8) KÖHLER, E.
1934. UNTERSUCHUNGEN ÜBER DIE VIRUSKRANKHEITEN DER KARTOFFEL. III. WEITERE VERSUCHE MIT VIREN AUS DER MOSAIKGRUPPE. Phytopath. Ztschr. 7: 1–30, illus.
(9) KOLMER, J. A., and TUFT, L.
1942. CLINICAL IMMUNOLOGY BIOTHERAPY AND CHEMOTHERAPY IN THE DIAGNOSIS, PREVENTION AND TREATMENT OF DISEASE. 941 pp., illus. Philadelphia and London.
(10) KUNKEL, L. O.
1934. STUDIES ON ACQUIRED IMMUNITY WITH TOBACCO AND AUCUBA MOSAICS. Phytopathology 24: 437–466, illus.
(11) LENNETTE, E. H., and HORSFALL, F. L., JR.
1941. STUDIES ON INFLUENZA VIRUS. THE COMPLEMENT-FIXING ANTIGEN OF INFLUENZA A AND SWINE INFLUENZA VIRUSES. Jour. Expt. Med. 73: 581–599.
(12) LESLEY, J. W., and WALLACE, J. M.
1938. ACQUIRED TOLERANCE TO CURLY TOP IN THE TOMATO. Phytopathology 28: 548–553, illus.
(13) PRICE, W. C.
1932. ACQUIRED IMMUNITY TO RING-SPOT IN NICOTIANA. Boyce Thompson Inst. Contrib. 4: 359–403, illus.
(14) ———
1936. VIRUS CONCENTRATION IN RELATION TO ACQUIRED IMMUNITY FROM TOBACCO RING SPOT. Phytopathology 26: 503–529, illus.
(15) ———
1940. ACQUIRED IMMUNITY FROM PLANT VIRUS DISEASES. Quart. Rev. Biol. 15: 338–361, illus.
(16) RIVERS, T. M.
1928. SOME GENERAL ASPECTS OF FILTERABLE VIRUSES. *In* Rivers, T. M. Filterable Viruses. 428 pp., illus. Baltimore.
(17) ———
1942. IMMUNITY IN VIRUS INFECTIONS. Science (n. s.) 95: [107]–112.
(18) SALAMAN, R. N.
1936. IMMUNITY TO VIRUS DISEASES IN PLANTS. Third Internatl. Cong. Compar. Pathol. Rpt. 1: 167–178.
(19) VALLEAU, W. D.
1935. DO TOBACCO PLANTS RECOVER FROM AND DEVELOP IMMUNITY TO RINGSPOT? Ky. Agr. Expt. Sta. Res. Bul. 360, pp. [181]–191, illus.
(20) ———
1941. EXPERIMENTAL PRODUCTION OF SYMPTOMS IN SO-CALLED RECOVERED RING-SPOT TOBACCO PLANTS AND ITS BEARING ON ACQUIRED IMMUNITY. Phytopathlogy 31: 522–533, illus.
(21) WALLACE, J. M.
1939. RECOVERY FROM AND ACQUIRED TOLERANCE OF CURLY TOP IN NICOTIANA TABACUM. Phytopathology 29: 743–749, illus.
(22) ———
1940. EVIDENCE OF PASSIVE IMMUNIZATION OF PLANTS FROM CURLY TOP (Abstract) Phytopathology 30: 26–27.
(23) ———
1940. EVIDENCE OF PASSIVE IMMUNIZATION OF TOBACCO, NICOTIANA TABACUM, FROM THE VIRUS OF CURLY TOP. Phytopathology 30: 673–679, illus.
(24) WINGARD, S. A.
1928. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. Jour. Agr. Res. 37: 127–153, illus.
(25) ZINSSER, H.
1932. ON POSTULATES OF PROOF IN PROBLEMS OF THE BACTERIAL LIFE CYCLE. Science (n. s.) 75: 256–258.
(26) ——— ENDERS, J. F., and FOTHERGILL, L. D.
1939. IMMUNITY PRINCIPLES AND APPLICATION IN MEDICINE AND PUBLIC HEALTH. (Ed. 5, Resistance to Infectious Diseases.) 801 pp., illus. New York.

○

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., SEPTEMBER, 15, 1944 No. 6

MOISTURE RETENTION BY SOME IRRIGATED SOILS AS RELATED TO SOIL-MOISTURE TENSION[1]

By L. A. RICHARDS, *senior soil physicist*, and L. R. WEAVER, *agent, United States Regional Salinity Laboratory, Riverside, Calif., Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

The processes of depletion and replenishment of soil moisture have received considerable attention from agricultural scientists because of the dependence of plant growth on the soil-moisture supply. The maximum amount of moisture that can be stored in soil in the field and the degree of dryness to which plants can reduce the moisture content of soil are the limits that determine the range of moisture available to plants. Numerous single-valued soil-moisture constants, such as moisture-holding capacity, moisture equivalent, field capacity, and the various wilting percentages, have been used for indicating the capacity of soils to retain water. The possible advantages of expressing moisture retention in terms of the physical condition of the moisture in soil or in terms of the security with which the water is retained as expressed by some energy or thermodynamic scale have long been considered (*4*).[2]

Some of the scales that have been proposed for this purpose will be discussed, but it is the main object of this paper to present data on the relation between the equivalent negative pressure or tension in the soil water and the moisture content for 71 soil samples collected by Furr and Reeves. On these samples the collectors[3] made careful determinations of the moisture equivalent, the first permanent wilting percentage, and the ultimate wilting percentage, and stated:

> With few exceptions the soil samples were taken from the top foot of soil, and most of them were from cultivated, irrigated orchards or fields. A few samples were from uncultivated desert or brushlands. The samples were air-dried and screened through a round-hole 2-mm. screen.

APPARATUS AND PROCEDURE

The pressure-membrane apparatus (*12*) was used to obtain moisture data at tensions above 1 atmosphere. This is a modification of ultrafiltration apparatus which has been used by Woodruff (*21*) for the same purpose. The extraction cells were the same as those already described (*12*), consisting of a Visking[4] membrane supported on a brass screen and plate with a cylindrical soil chamber 29 cm. in diameter and 1.3 cm. high. Moisture was extracted by water-pumped nitrogen supplied from a storage cylinder.[5]

---

[1] Received for publication March 3, 1943. Cooperative investigation of the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, Riverside, Calif.; 11 Western States; and the Territory of Hawaii.

[2] Italic numbers in parentheses refer to Literature Cited, p. 234.

[3] FURR, J. R., and REEVES, J. O. THE RANGE OF SOIL MOISTURE PERCENTAGES THROUGH WHICH PLANTS UNDERGO PERMANENT WILTING IN SOME SOILS FROM SEMIARID IRRIGATED AREAS. 1942. [Unpublished manuscript.]

[4] The Visking Corporation, Chicago, Ill.

[5] A small refrigeration compressor, on which tests have just been completed, has been found to supply ample quantities of compressed air for pressure-membrane work and has been operated at pressures up to 420 pounds in.$^{-2}$.

Journal of Agricultural Research, Washington, D. C.

Vol. 69, No. 6 Sept. 15, 1944 Key No. G-1309

During moisture extraction most soils undergo a certain amount of shrinkage that tends to pull the soil out of contact with the membrane. To prevent this, a soft-rubber diaphragm actuated by a 5 pound per square inch pressure differential was used to hold the soil against the membrane. This pressure differential was obtained by inserting in the line between the pressure source and the soil chamber a mercury U-tube fitted with a bypass valve. The diaphragm pressure was obtained from the pressure-inlet side of the U-tube and was applied by closing the bypass valve and venting the soil chamber. To prevent undesired compacting and puddling effects on wet soils, the diaphragm pressure was not applied until the moisture content of the sample was reduced to somewhere near the 1-atmosphere percentage. In future work it is contemplated that the mercury U-tube will be replaced by a water U-tube.

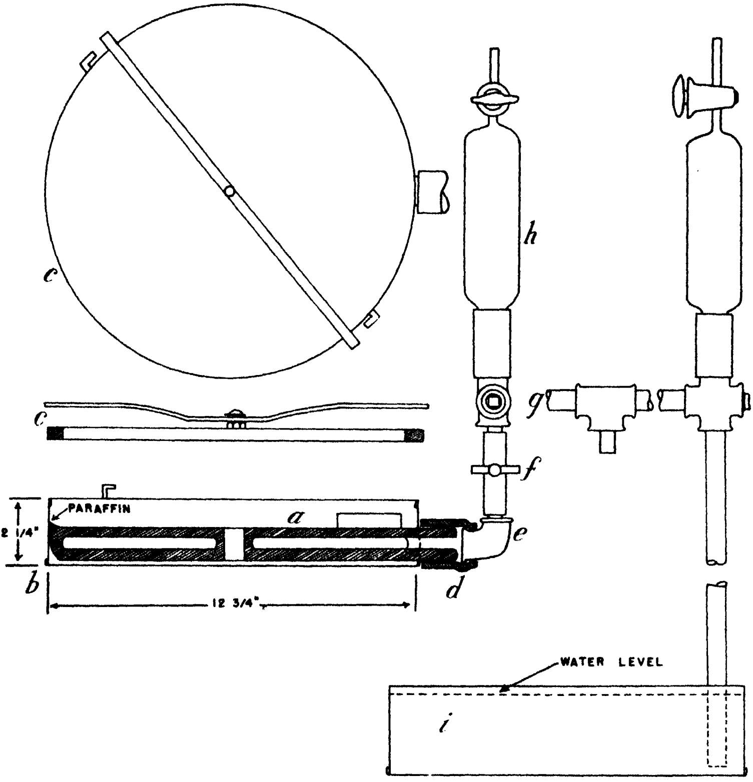


FIGURE 1.—Suction-plate apparatus for determining single moisture-retention values for soils in the 1-atmosphere tension range: *a*, Ceramic cell with porous upper surface; *b*, galvanized-iron box; *c*, box lid with spring clamp and sponge-rubber gasket; *d*, 7/8-inch automobile radiator hose; *e*, 3/8-inch galvanized pipe L with 3/8- to 1/4-inch pipe bushing, which has a section of 5/16-inch copper tubing soldered in place; *f*, 3/8-inch (inside diameter) vacuum rubber tubing with pinch clamp; *g*, manifold for making connection to a number of cells; *h*, glass air trap; *i*, water reservoir.

Moisture-retention data at tensions between zero and 1 atmosphere were obtained either with pressure-plate or suction-plate apparatus. The pressure-plate apparatus is identical in principle with the pressure-membrane apparatus but makes use of a porous ceramic plate instead of the cellulose membrane. The suction-plate apparatus used is shown in figure 1. The porous ceramic cell[6] is of single-piece construction and is mounted in a galvanized-iron box. The lid is fitted with a sponge-rubber gasket and a spring clamping bar. The cell spout extends from a hole in the box and connects through various fittings to the manifold. This manifold has connections for as many of the suction-plate units as are needed and slopes upward toward the pipe cross and the separatory funnel that is used as an air trap. A pipe extends downward from the air trap to a free water surface, the elevation of which determines the negative pressure at the porous surface. The suction, of course, can be produced when desired by a controlled vacuum system.

The procedure for obtaining all of the moisture-retention data presented in this paper was as follows: A layer of screened, air-dried soil was placed on the porous moisture-extracting surface; the soil was wet thoroughly with an excess of distilled water, and then the moisture was extracted until the moisture tension in the soil increased to a constant predetermined equilibrium value and moisture outflow from the sample ceased. For the determination of single moisture-retention values, a number of soil samples were brought to equilibrium in the same extraction cell, the moisture-content determinations being made by drying to constant weight at 105° C. Moisture retention was expressed as percentage of dry weight so as to be comparable with moisture-equivalent and wilting data.

The soil samples were kept separate on the porous plate by placing them in thin-walled brass rings 5.4 cm. in diameter and 1 cm. high. This permitted 12 to 14 determinations per ground cell. Rings 2 cm. high can be used when larger soil samples are desired.

When moisture-retention curves were desired, the entire pressure-membrane or pressure-plate cell was loaded with one soil sample. The moisture percentage at any desired number of equilibrium-tension values can be calculated from the combined record of the moisture extracted at each succeeding tension increment, the final moisture content, and the total dry weight of the sample.

The amount of moisture a soil will retain at a given tension depends somewhat on the time allowed for wetting the air-dried sample. Some sandy soils show no increase in moisture retention for wetting time beyond 15 minutes, but some fine-textured soils require as much as 18 to 24 hours before the moisture retained is independent of the wetting time. An overnight wetting time of 16 to 18 hours was used for the determinations reported in this paper.

For all except very impermeable soils, 4 to 6 hours is ample time for a 1- to 2-cm. layer of saturated soil to come to equilibrium after the pressure differential is applied to the porous ceramic plates used. The time required for outflow equilibrium in the pressure-membrane apparatus is indicated by the curves in figure 8, which will be discussed later.

---

[6] Ground cell K 939-B, General Ceramics and Steatite Company, Keasbey, N. J.

Unless otherwise indicated, all the soil-moisture tension data presented in this paper were obtained at a temperature of 21° C., and the following pressure equivalents were used: 1 atmosphere=1.013×10^6 dyne cm.$^{-2}$=14.71 pounds in.$^{-2}$=76.39 cm. of mercury=1,036 cm. of water=34.01 feet of water.

FIFTEEN-ATMOSPHERE PERCENTAGE AND THE WILTING RANGE

For the soils used in the first tests of the pressure-membrane apparatus it was found that an extraction pressure of 16 atmospheres reduced the moisture content slightly below the wilting percentage, as determined with sunflowers by Eaton and Horton (*6*). In view of this preliminary experience it was decided to determine the 15-atmosphere percentage for the soil samples collected by Furr and Reeves. Table 1 gives the moisture equivalent, the first permanent wilting percentage, and the ultimate wilting percentage as determined by Furr and Reeves, and in addition gives the moisture retained by these soils at five soil-moisture tension values.

TABLE 1.—*Moisture* [1] *retained by soils after moisture-extracting treatments*

Soil type	Soil accession No.	Moisture equivalent	First permanent wilting	Ultimate wilting	Moisture retained at indicated soil-moisture tension				
					Centimeters of water				Atmospheres
					250	345	440	518	15
Coarse soils:									
Washed and screened sand, 10-20 mesh	57	*Percent* 1.3	*Percent* 1.2	*Percent* 0.7	*Percent* 1.2	*Percent* 1.2	*Percent* 1.2	*Percent* 1.1	*Percent* 0.8
Washed and screened sand, 20-30 mesh	56	1.4	1.3	.7	1.3	1.2	1.3	1.2	.9
Washed and screened sand, below 30 mesh	55	1.9	1.4	.9	1.9	1.5	1.7	1.6	1.0
Tujunga sand	52	2.6	1.8	1.2	2.3	2.1	2.2	2.1	1.4
Hanford sand	51	4.8	2.9	1.9	5.0	4.5	4.3	4.1	2.2
Ramona sand	54	5.0	2.9	2.2	5.2	4.4	4.5	4.2	2.0
Indio loam	76	5.2	2.6	1.6	5.3	4.6	4.3	3.8	1.6
Indio very fine sand	48	5.8	3.2	2.0	5.9	5.0	4.7	4.1	1.9
Dune sand	53	6.5	2.6	1.9	6.6	5.6	5.4	5.2	2.9
Superstition (leached) sand	50	6.6	2.4	1.5	6.5	------	4.8	4.4	1.9
Holland sandy loam	41	6.9	4.2	3.2	7.6	6.4	6.2	5.5	2.7
Indio very fine sandy loam	70	7.3	3.2	1.9	8.2	6.9	6.4	5.7	2.2
Indio loam	75	7.4	2.8	1.9	7.5	6.2	6.2	5.1	1.9
Placentia sandy loam	43	7.4	3.9	3.2	8.1	6.7	6.4	6.0	2.5
Indio fine sandy loam (6 feet)	82	7.7	3.9	2.5	9.2	8.0	7.5	6.6	2.7
Hanford fine sand	47	8.0	3.8	2.5	8.8	7.6	7.3	6.8	2.3
Fresno fine sandy loam	40	8.9	3.7	2.5	10.2	8.5	8.1	7.5	2.3
Ramona fine sandy loam	37	9.2	5.2	3.7	10.1	8.3	8.3	7.8	3.5
Tujunga Stony sand	58	9.8	5.2	3.8	10.2	9.1	8.8	8.1	4.3
Indio fine sandy loam (5 feet)	81	9.9	5.2	3.2	12.3	10.7	10.0	8.8	3.1
Placentia clay loam	17	10.9	6.0	4.7	11.8	10.0	9.8	8.7	5.6
Hanford gravelly sandy loam	26	11.3	5.4	3.5	12.6	9.8	9.5	9.0	3.5
Hanford sandy loam	42	12.4	4.8	3.6	14.2	11.7	10.6	9.7	2.9
San Joaquin sandy loam	46	12.5	5.9	4.1	14.1	12.1	12.2	11.6	4.3
Greenfield loam	N4	12.7	6.3	5.1	17.3	14.5	14.2	13.1	5.4
Tujunga fine sandy loam	39	13.2	5.5	4.1	20.3	15.6	13.8	12.2	3.5
Yolo loam	31	14.3	8.4	6.7	15.1	12.6	12.7	11.6	7.1
Hanford loam	29	14.5	6.0	4.0	16.9	14.0	13.2	11.8	4.4
Placentia loam	28	14.8	7.7	5.3	16.9	14.0	13.4	12.6	5.7
Coachella very fine sand	49	15.2	5.7	4.0	18.1	14.5	13.2	12.1	3.8
Indio loam (4 feet)	74	15.4	6.1	3.3	18.0	------	14.5	13.2	3.8
Altamont clay	2	15.6	6.8	5.2	19.4	15.4	14.7	13.4	5.7
Yolo fine sandy loam	38	15.4	8.3	6.2	14.7	12.6	12.1	12.0	5.5
Total [2]	------	279.6	137.8	98.0	318.3	265.3	254.2	233.1	99.6

[1] Expressed on a dry-weight basis.

[2] Omitting soils 50 and 74.

TABLE 1.—*Moisture retained by soils after moisture-extracting treatments*—Con.

Soil type	Soil accession No.	Moisture equivalent	First permanent wilting	Ultimate wilting	Moisture retained at indicated soil-moisture tension				
					Centimeters of water				Atmospheres
					250	345	440	518	15
Fine soils;		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Madera sandy loam	45	16.0	6.6	4.8	16.0	13.2	12.9	11.8	4.5
San Joaquin loam	33	16.2	8.1	6.3	18.0	15.1	15.4	14.4	6.5
Ramona loam	27	16.6	8.3	5.6	18.7	15.4	15.1	14.3	6.0
Indio fine sandy loam (5 feet)	79	16.9	6.8	3.8	19.9	17.0	16.0	14.9	4.6
Indio fine sandy loam (1 foot)	77	17.5	5.9	3.5	21.9	17.7	16.3	14.7	4.2
Madera loam	32	17.6	9.9	7.7	22.1	18.0	18.5	16.6	8.4
Hanford fine sandy loam	36	17.8	6.7	4.7	22.5	18.5	16.9	15.2	4.3
Altamont clay loam	14	18.4	10.0	8.8	19.0	16.1	16.4	15.3	9.1
Indio very fine sandy loam (5 feet)	69	19.0	5.6	4.0	21.2	18.8	17.7	15.9	4.8
Ramona clay loam	15	19.0	9.3	6.3	23.1	19.4	18.9	17.5	6.7
Indio loam (3 feet)	73	20.2	7.0	4.2	24.2	-------	19.8	17.9	5.0
Antioch silty clay loam	22	20.9	10.7	9.0	25.2	20.8	20.0	17.9	9.5
Chino loam	30	21.0	10.2	7.5	23.6	19.7	19.8	18.4	8.0
Indio very fine sandy loam (4 feet)	68	21.2	6.9	4.3	23.9	-------	20.1	18.3	5.2
Yolo clay loam	16	21.3	12.3	10.4	23.7	20.4	19.8	18.1	10.2
Madera clay loam	18	21.7	11.7	8.2	24.0	21.3	21.4	20.2	8.8
Indio fine sandy loam (4 feet)	80	22.8	8.8	5.3	27.0	23.7	22.5	20.9	6.4
Indio clay loam	85	22.9	8.9	5.9	29.2	24.6	23.0	20.6	6.5
Indio loam (1 foot)	71	23.2	8.0	5.1	27.1	22.8	21.9	20.0	6.1
Montezuma clay	4	23.4	13.2	10.5	22.8	20.2	19.8	17.6	11.3
Hanford silty clay loam	25	24.4	14.3	12.1	29.5	24.3	25.4	22.8	13.2
Montezuma clay	8	25.9	13.3	10.1	30.0	25.9	27.2	24.9	12.7
Indio fine sandy loam (2 feet)	78	26.1	11.5	5.8	31.2	-------	26.1	24.1	7.0
Fresno loam	34	26.8	14.2	9.6	34.4	30.5	29.3	27.2	10.7
Dublin clay	1	27.4	16.4	13.2	30.7	27.6	27.3	24.0	14.2
Chino silty clay loam	23	27.6	14.4	10.4	30.5	26.8	25.9	24.3	11.0
Antioch clay	5	29.5	17.8	15.5	38.6	28.5	29.0	26.9	16.5
Indio loam (2 feet)	72	30.3	8.8	5.7	37.5	32.5	31.4	27.9	6.7
Yellow clay loam (Stevens No. 2)	20	31.0	20.2	16.9	34.5	31.2	31.1	29.7	18.4
Ducor clay	10	31.2	17.1	14.4	35.3	32.0	32.0	28.7	16.5
Merced loam	35	32.0	16.4	11.7	39.5	36.4	34.9	32.5	13.4
Diablo clay	3	34.1	18.2	15.0	33.1	34.0	33.9	31.0	17.7
Chino silty clay loam (heavy phase)	24	37.1	22.4	19.2	40.5	35.6	36.5	33.9	21.1
Chino silty clay	12	37.6	23.2	20.1	42.8	40.8	39.5	36.1	21.9
Portersville clay	7	40.2	21.2	17.1	46.4	41.5	43.5	41.2	23.2
Olympic clay	9	41.8	24.4	19.9	48.0	42.6	43.1	40.2	23.6
Chino silty clay loam	13	43.2	23.3	14.4	53.1	48.9	47.0	44.0	15.0
Yolo clay	6	45.9	29.6	23.6	52.3	45.1	48.1	43.5	26.2
Total [3]	-------	918.2	476.2	366.3	1,061.7	926.4	917.4	843.1	407.9

[3] Omitting soils 68, 73, and 78.

Figure 2 is a scatter diagram showing the relation of the 15-atmosphere percentage to the wilting range. Vertically placed pairs of points give the position of the ultimate and the first permanent wilting percentage of each soil on the moisture scale. The intersection of a vertical line connecting a pair of points with the diagonal locates the 15-atmosphere percentage with respect to the wilting range. It is seen that with but comparatively few exceptions for the soils tested the 15-atmosphere percentage lies in the wilting range. It is estimated that if the complete root system of the wilted plant had been removed from the soil the wilting percentages would have been reduced by 0.2 to 0.3 percent. This reduction would bring the 15-atmosphere percentage of all but 7 of the 71 soils within the wilting range.

The location of the 15-atmosphere percentage in the wilting range may be indicated by another method. The soils in table 1 have been divided into a coarse group (moisture equivalent less than 16 percent) and a fine group (moisture equivalent 16 percent or higher). Totals of the data are given for all of the soils in the two groups except the five for which the data are incomplete. From these totals it is seen that for the coarse group the 15-atmosphere percentage lies in the wilting range and only 0.04 of the wilting range from the ultimate wilting percentage, and for the fine group the 15-atmosphere percentage is 0.38 of the wilting range from the ultimate wilting percentage.

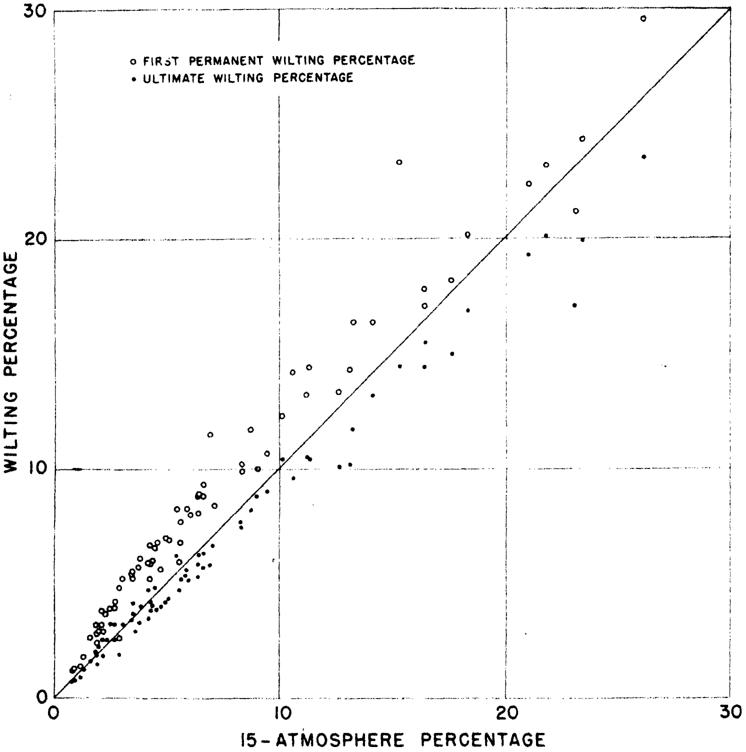


Figure 2.—A scatter diagram showing the relation of the 15-atmosphere percentage to the wilting range.

The problem of getting representative samples was given particular attention. Subsamples were taken in such a way as to cause a minimum of particle-size segregation after "pulling" all of the original sample on a Koroseal-coated cloth. The 15-atmosphere percentages shown in figure 2 and table 1 are the average of triplicate determinations. The coefficient of variation[7] for the determinations was larger for the coarse soils, running as high as 5.0 in some cases, but the average coefficient of variation for the whole group of soils was 1.46.

---

[7] Standard deviation expressed as percentage of mean.

Determination of the 15-atmosphere percentages was made on 21 individual soil samples in one extraction unit at one time by placing the samples in rubber rings 4.1 cm. in diameter and 1 cm. high. The rings were cut from the inner tube of a bicycle tire. The samples were covered with individual squares of waxed paper when placed in the cell so as to minimize vapor losses during transfer to moisture boxes at the end of a run.

SOIL-MOISTURE TENSION AND THE MOISTURE EQUIVALENT

The scatter diagrams in figure 3 show the relation between moisture equivalent as determined by Furr and Reeves and the moisture retained when these soils are wetted and then brought to equilibrium on the suction plate at the four tension values 250, 345, 440, and 518 cm. of water. The determinations were made in triplicate. The coefficients of variation calculated for the 518-cm. determinations were not related to texture and had an average value of 1.50. It is evident that on an average there is a fairly close relation between moisture equivalent and the moisture retained at the moisture tension of 345 cm. of water (⅓ atmosphere).

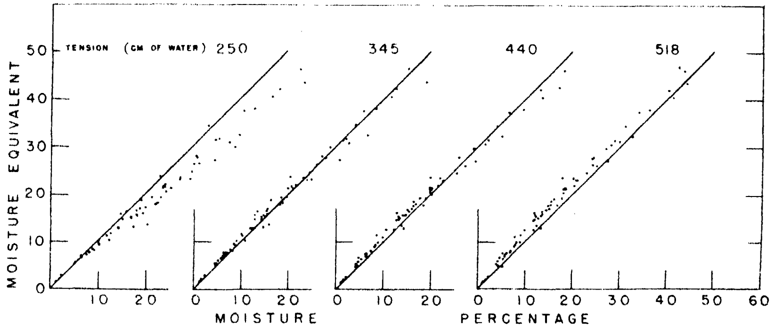


FIGURE 3.—Scatter diagrams showing the relation of the moisture equivalent to the moisture retained by the same group of soils at soil-moisture tensions of 250, 345, 440, and 518 cm. of water. Comparison of the change produced by the various tension increments is aided by the 45° reference lines.

The totals in table 1 indicate that the ⅓-atmosphere percentage is slightly lower than the moisture equivalent for the coarse soils and slightly higher than the moisture equivalent for the fine soils.

It will be noted that there are inconsistencies in table 1. At moisture tensions of 345 and 440 cm. of water, a lower moisture content was found at the lower tension for some of the soils. The authors feel that these inconsistencies were caused by sampling error and do not indicate an inherent lack of precision in the suction-plate procedure, since there was excellent agreement among the triplicate determinations (coefficient of variation, about 1.5). Chronologically the moisture-retention values for the soils at 345 cm. of water tension were the last data determined in the table, and although considerable care was used in subsampling, the inconsistencies in the table seem to indicate that repeated subsampling shifted the texture of some of the stock samples toward lower moisture retention.

TEMPERATURE EFFECT ON MOISTURE RETENTION

Because of the evidence in the literature that temperature has a definite effect on moisture retention, it was felt that part of the scatter in figures 2 and 3 might arise from the fact that the various moisture-equivalent and wilting determinations were made at different temperatures. To get information on this point, 12 soils, covering a wide texture range, were selected from the laboratory-stock samples and the Furr and Reeves collection. Triplicate determinations of the ½-atmosphere percentage and the 15-atmosphere percentage were made for each soil at 4 different temperatures. The results of these measurements are given in table 2 and figure 4. Slope and intercept values for least-square straight lines are given in table 2, and these lines are shown in figure 4 along with the experimental points. With but one exception the slopes were negative, as would be expected from the effect of temperature on surface tension. The change in moisture retention per degree of change in temperature increased from coarse to fine texture, but appeared not to be linearly related to the moisture retention of the various soils at any given temperature and tension.

TABLE 2.—*Effect of temperature on moisture retained at ½ and 15 atmospheres*

Soil type	Accession No.	Moisture retained at ½ atmosphere and indicated temperature (°C.)					$\frac{dPw}{dt}$[1]	Moisture retained at 15 atmospheres and indicated temperature (°C.)					$\frac{dPw}{dt}$[1]
		[1] 0	12.2	21.2	29.7	37.2		[1] 0	12.4	21.1	29.5	37.5	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Tujunga sand	52	2. 76	2. 47	2. 42	2. 23	1. 99	-. 0193	1. 66	1. 46	1. 25	1. 23	1. 00	-. 0167
Placentia sandy loam	43	6. 10	5. 94	5. 80	5. 63	5. 60	-. 0141	3. 49	3. 15	3. 03	2. 78	2. 55	-. 0244
Hanford gravelly sandy loam	26	8. 46	8. 28	8. 50	8. 30	8. 46	. 0041	3. 88	3. 45	3. 49	3. 31	2. 82	-. 0245
Placentia loam	28	12. 62	12. 48	12. 41	12. 16	12. 24	-. 0118	6. 36	5. 79	5. 72	5. 39	[2] 4. 88	-. 0363
Sagemoor fine sandy loam	S-40-10	11. 90	11. 68	11. 64	11. 28	11. 35	-. 0163	6. 92	6. 18	5. 80	5. 25	4. 79	-. 0563
Indio very fine sandy loam	S-40- 4	18. 99	18. 31	17. 86	17. 98	16. 87	-. 0494	6. 80	6. 49	6. 23	6. 00	5. 83	-. 0264
Chino loam	30	18. 78	18. 45	18. 39	17. 78	17. 96	-. 0252	8. 91	8. 20	7. 94	7. 41	6. 94	-. 0514
Billings clay	S-40- 7	22. 92	22. 15	20. 66	20. 82	19. 81	-. 0823	9. 41	8. 77	8. 56	8. 13	7. 66	-. 0448
Altamont clay loam	14	15. 36	15, 32	15. 28	14. 86	15. 28	-. 0070	10. 28	9. 27	9. 46	8. 74	[2] 7. 90	-. 0572
Meloland clay	S-40- 2	28. 25	28. 00	27. 60	27. 51	27. 32	-. 0257	17. 11	15. 63	14. 93	14. 13	12. 87	-. 1082
Antioch clay	5	28. 29	27. 67	26. 67	26. 31	26. 00	-. 0649	20. 33	18. 28	16. 79	15. 76	14. 06	-. 1634
Yolo clay	6	44. 73	44. 37	41. 80	42. 83	41. 81	-. 0808	30. 75	28. 20	26. 04	24. 96	22. 69	-. 2102

[1] Values taken from least-square equation having the form $Pw=a+bt$, where Pw represents moisture percentage, t represents temperature, $a=Pw$ for $t=0$ and $b=dPw/dt$.

[2] Values calculated by C. H. Wadleigh, using the missing-plot technique.

SOIL-MOISTURE RETENTION CURVES

Curves showing the relation between the security with which water is held by soil and the amount of water in the soil are being increasingly used in soils work because of their relation to pore-size distribution, structure, and the nature and extent of the soil surface (*5*, *9*).

The curves shown in figure 5 were obtained on air-dried and screened soil samples, this being the normal preparation for moisture-equivalent and wilting-point determinations. The jog in the curves at the 1-atmosphere percentage occurs at the juncture between pressure-plate and pressure-membrane data. With one exception for the curves shown, the discrepancy is less than 0.5 percent and is

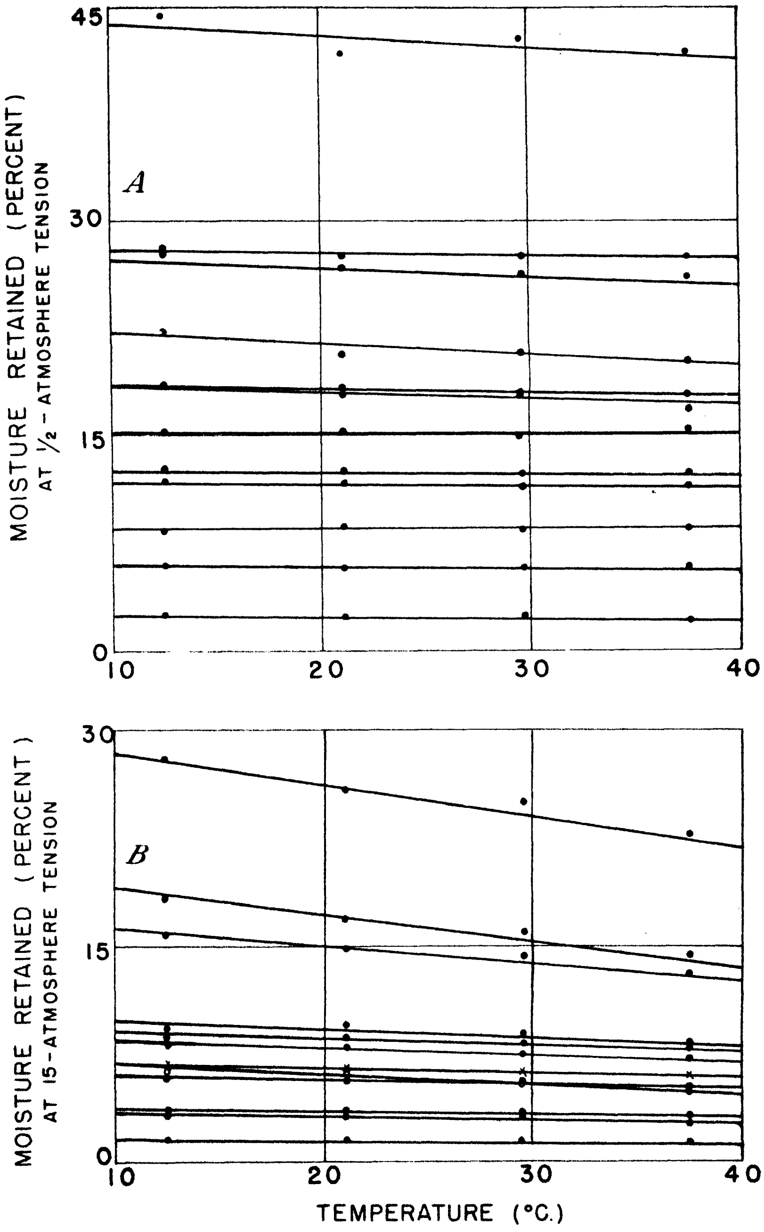


FIGURE 4.—Effect of temperature on the moisture retained by a group of soils at one-half atmosphere (*A*) and 15 atmospheres (*B*) of soil-moisture tension.

believed to be due to soil differences or other imperfection in experimental procedure and is not to be attributed to the change from the ceramic plate to the cellulose membrane. Curves obtained on duplicate samples agree closely, if care is taken to insure that the samples are as nearly as possible identical. The high-tension parts of the curves were obtained by spreading approximately 420 gm. of

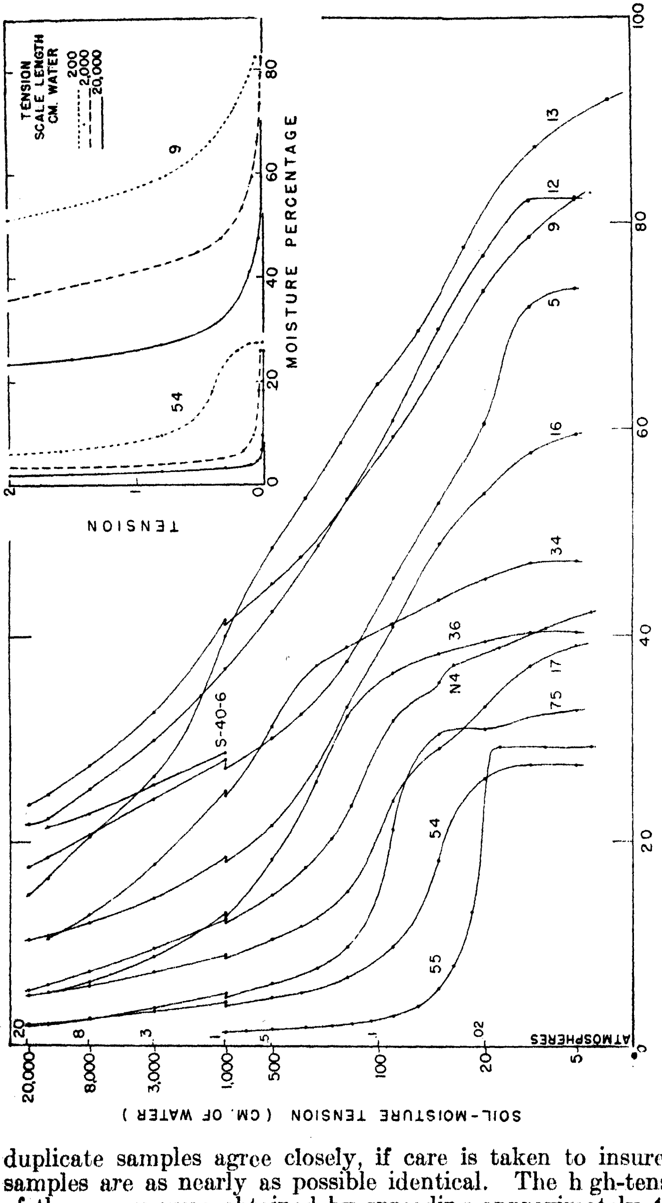


FIGURE 5.—Moisture-retaining properties of soils. The logarithmic scale is helpful for giving a visual comparison over a wide tension range. To show the effect of the kind of scale on the apparent slope of the retention curve, data from the logarithmic curves for soils 54 and 9 are each replotted on three different linear scales in the upper right-hand corner.

dry soil on a circular Visking membrane 29 cm. in diameter, whereas for the low-tension data approximately 55 gm. of soil was spread on a ceramic plate 8.9 cm. in diameter.

A number of interesting facts are brought out by the curves plotted on the logarithmic scale. The marked differences in slope and the crossing of the curves in the high-tension range discourage any attempt to find a general relation between moisture equivalent and the wilting percentage. It is apparent that some of the curves that coincide at the 15-atmosphere tension are widely separated at lower tensions. Since the position and slopes of the curves at the higher tensions are determined largely by the kind and amount of soil colloid, it is likely that moisture-retention data on the extracted colloid will have some use in colloid identification work. The Aiken soil is known to have predominantly kaolinitic colloid and in the field has a very narrow range of moisture available for plants, as indicated by the steepness of the moisture-retention curve for the Aiken soil, which is marked S-40-6 in figure 5.

The smooth curves between the experimental points might be somewhat altered if additional points were determined. In the low-tension range the curves are shifted considerably by small changes in the history or physical condition of the sample, but this sensitivity increases the usefulness of the curves for studying the stability of structure as proposed by Childs (*5*). Since the determinations were made on air-dried and screened samples, the curves differ from those that would have been obtained with field structure.

The moisture-retention data shown by the logarithmic curves for soils 54 and 9 in figure 5 are replotted in the upper right-hand corner of the figure on linear scales. This was done to illustrate the effect of the method of plotting on the apparent shape of the curves. The same moisture scale was used for all of the inset curves, but the dotted curves show the retention data for 0-200 cm. of water; the broken curves, for 0-2,000 cm.; and the solid-line curves, for 0-20,000 cm. It is at once apparent that the position of the "knee" of the curve on the moisture axis depends on the scale and the amount of the retention curve being examined. The appearance of rapid change in the slope of the curve in this region has no special biological or physical significance as far as the moisture-retaining characteristics of the soil are concerned.

SOIL-MOISTURE RELATIONS IN THE 1-ATMOSPHERE RANGE

The moisture equivalent has been widely used as an index of moisture retention by soil, and it is interesting to see how this constant is related to the moisture-retention curve.

The work of Schaffer, Wallace, and Garwood (*16*) indicates that the pressure in the soil moisture is zero at the periphery of the moisture-equivalent centrifuge sample. From this boundary condition, the soil-moisture-tension values at successive 1-mm. distances from the periphery or moisture-outflow surface of the centrifuge sample may be calculated (*15*) from the equation $T=(\omega^2/2g)(r_1^2-r_2^2)$ and are found to be successively 0, 101, 201, 301, 400, 498, 596, 692, 789, 884, and 979 cm. of water. Quantities represented by the symbols in the equation are T, tension; ω, angular velocity of centrifuge; g, acceleration of gravity; r_1 and r_2, distances from center of rotation. The depths of the soil after centrifugation for a number of 30-gm. moisture-equivalent samples were carefully measured, and the majority were found to range from 9 to 10 mm. deep.

TABLE 3.—*Moisture-retention data as related to moisture equivalent, tensiometer range, and field capacity*

Item	Soil accession No.											
	55	54	75	17	N4	36	16	34	5	12	9	13
(1) Moisture equivalent	1.9	5.0	7.4	10.9	12.7	17.8	21.3	26.8	29.5	37.6	41.8	43.2
(2) Average *Pw*[1] from curves in figure 5 over 0.1- to 1.0- atmosphere tension range	1.6	5.0	6.5	10.9	15.5	19.6	23.0	31.5	30.6	43.3	45.9	48.9
(3) Ratio between numbers in item 2 and item 1	.84	1.00	.88	1.00	1.22	1.10	1.08	1.16	1.04	1.15	1.10	1.13
(4) Tension value on curve at *Pw* in item 2	450	400	420	410	420	442	415	483	450	450	450	475
(5) *Pw* at 40 cm. of water tension	5.5	18.1	30.4	29.0	35.4	38.2	48.9	43.4	52.8	69.8	68.3	72.7
(6) Ratio of *Pw* at 40 cm. of water tension to the moisture equivalent multiplied by 2	1.42	1.81	2.05	1.33	1.39	1.07	1.15	.81	.89	.93	.82	.84
(7) Difference between *Pw* at 0.1- and 0.85-atmosphere tension	1.25	4.5	11.6	12.1	16.3	21.0	19.6	13.8	12.8	19.3	15.3	21.1
(8) Difference between *Pw* at 0.1- and 15-atmosphere tension	1.7	6.7	14.9	15.7	23.4	31.0	28.4	29.8	23.8	36.1	33.7	49.4
(9) Ratio between numbers in item 7 and item 8	.74	.67	.78	.77	.70	.68	.69	.46	.54	.53	.45	.43

[1] The symbol *Pw* represents percentage of water in the soil expressed on a dry-weight basis.

It is difficult to say exactly where in the centrifuge soil-cup system the zero-pressure boundary condition applies. If this is taken to be the surface between the screen and the centrifuge case, then about 1 mm. is taken up by the screen and the filter paper, and on the basis of the above calculations this would give a tension of 100 cm. of water at the outer surface of the soil. The moisture equivalent is the average moisture content for the whole centrifuge sample and, therefore, should be the average of moisture values taken from a retention curve at points corresponding to the tension and packing at successive 1-mm. layers of the sample. Since the departure from linear tension distribution is small, it should be possible to approximate the moisture equivalent by averaging the moisture-content values on the moisture-retention curve for the centrifuged sample between the tension limits determined by the distances of the inner and outer soil boundary from the water-outflow surface. The average moisture percentage for the 100 to 1,000 cm. of water-tension range of each of the curves in figure 5 was determined, and these values are given in item 2 of table 3. The ratios of these average values to the corresponding moisture equivalents are given in item 3. The average value ratio is 1.06. These results are about what might be expected, since for most soils centrifugation produces denser packing and less pore space than existed in the uncentrifuged pressure-plate samples. The curves in figure 5, particularly at lower tensions, show higher moisture retention than would be found if the samples had been compacted in the centrifuge. The foregoing analysis, therefore, supports the view that the moisture equivalent is the average value over approximately the 0.1- to 1.0-atmosphere tension range for a moisture-retention curve that takes into account centrifuge packing effects.

It is becoming increasingly clear that the moisture equivalent cannot be generally used as an index of either the upper (*3, 11*) or the lower [8] (*20*) limit of moisture usable by plants in the field. Moisture equivalent has the advantage of being a definite reproducible quantity not too difficult to determine, but this is insufficient justification for its continued use provided something more closely related to the available moisture range can be found. It is apparent from figure 3 that moisture-retention values in the ¼- to ½-atmosphere ranges are too closely related to moisture equivalent to be of appreciably greater use or significance, except that (1) they are less expensive to determine and (2) they do represent a more definite physical property of the soil moisture. This latter is some advantage since it makes the determination independent of the kind of apparatus used, provided, of course, that the procedure does not alter the condition of the sample. As a substitute for moisture equivalent the ⅓-atmosphere percentage appears to merit some consideration, but the authors feel that the expression "moisture equivalent" should be used only in connection with determinations made with the Briggs and McLane equipment.

From tensiometer data now available for several soils it appears that field capacity may correspond to a tensiometer reading somewhere near 0.1 atmosphere, but there seems to be no distinctive feature of the tension-time curve following irrigation that can be associated with the condition of field capacity. If further field measurements should indicate that there is a certain tension range that approximates field capacity, it would be possible, by adjusting the height of the sample, the thickness of a standard porous pad under the sample, and the speed, to set up a centrifuge method that would give the average moisture percentage for any section of a moisture-retention curve. The fact that the field capacity depends on the nature and condition of the whole profile, including the initial moisture distribution, the moisture-transmitting properties of the soil, the moisture-retaining properties of the soil, and the amount of water applied, increases the difficulty of basing a field-capacity estimate on a soil sample isolated from the profile.

It might be expected that an estimate of field capacity could be more readily based on a soil sample having field structure than on one that is dried and screened, but the advantages of the latter for routine work are obvious. Centrifuge packing may partly overcome the structural disruption caused by screening, but the ratio of field capacity to moisture equivalent is considerably higher for coarse than for fine soils (*3, 11*). The possibility that a moisture-retention value at a lower tension than the ⅓-atmosphere percentage may be a better indication of field capacity is suggested by the fact that this tension empties a relatively larger fraction of the pore space for the coarse-textured soils than for the fine-textured soils. The numbers given in item 6 of table 3 were obtained by dividing the moisture retained at 40 cm. of water tension (item 5) by twice the moisture equivalent. These numbers when plotted against moisture equivalent correspond closely to Browning's (*3*) field-capacity moisture-equivalent ratio curve when the latter is corrected for the difference between the Gooch crucible and the standard moisture-equivalent procedure. This correspondence indicates that for these 12 soils half the water retained at 40 cm. of water tension by a sample that has been air-dried and screened closely approximates the field capacity as determined by Browning. This agreement may be only fortuitous, but it is possible that further work along this general line may yield a useful field-capacity index.

---

[8] See footnote 3, p. 215.

The curves in figure 5 give basis for an estimate of the fraction of the available range of moisture over which tensiometers can be used. Item 7 in table 3 gives the difference between the 0.1-atmosphere percentage and 0.85-atmosphere percentage, these being common limits between which field tensiometers (*13*) have been found to operate. The figures in item 8 are the difference between 0.1-atmosphere percentage and 15-atmosphere percentage and are a measure of the available range of moisture for the various soils. Item 9 gives the ratios of the numbers in item 7 to those in item 8 and indicates the fraction of the available range over which tensiometers can be used. This fraction is seen to vary from less than 0.5 in the fine soils to about 0.8 in the coarse soils. Under conditions of restricted drainage, this fraction is appreciably increased. For a soil having a permanent wilting percentage of 3.7, which was used in 20-gallon culture cans provided with free drainage, it has been found that the moisture range over which tensiometers operate comprises 0.9 of the available range.[9]

SOIL-MOISTURE TENSION IN THE WILTING RANGE

Since it is not yet possible to measure directly the soil-moisture tension in a sample of soil in the wilting range, some information on the range in tension that corresponds to the wilting range may be obtained indirectly by placing the wilting percentages on the moisture-retention curves (*14*, *21*).

In figure 6 the first permanent wilting percentage and the ultimate wilting percentage as determined by Furr and Reeves have been located on moisture-retention curves determined with the pressure-membrane apparatus. Broken lines indicate where the curves were extrapolated beyond the experimentally determined points. From these results it would seem that neither the first permanent wilting percentage nor the ultimate wilting percentage is closely related to soil-moisture tension.

To get information on the free energy, or pF, at wilting for the Furr and Reeves samples requires consideration of soluble salt content as well as soil-moisture tension. The osmotic concentration of the soil solutions at the wilting points could have been determined by measuring the freezing points on soil solutions extracted from samples in which the sunflowers were wilted. This was not done, but an estimate of the osmotic pressures involved can be obtained by another method. Column 2 of table 4 gives the moisture percentage of the soils at the beginning of the extraction process used for the determination of the curves in figure 6. The amounts of dissolved solids in the extracts were determined, and the soluble salt content of the soils, expressed as percentage on a dry basis, is given in column 3. Column 4 gives the osmotic concentration of the extracted solution. This latter was obtained by dividing the electrical conductivity expressed in micromhos (at 25° C.) by 28.5.[10] Multiplying these values by the ratios of the initial extraction percentage to the wilting percentages gives an indication of the osmotic pressures at the wilting points.[11] The remainder of the table gives the soil-moisture tension, the osmotic concentration, and the sum of these two at first permanent wilting and ultimate wilting.

---

[9] Correspondence with C. S. Scofield concerning work in progress at the Rubidoux Laboratory, Riverside, Calif.

TABLE 4.—*Soil-moisture tension and osmotic concentration of the soil solution at wilting*

Soil accession No.	P_w before extraction	Soluble salt in soil	Osmotic concentration of extract	First permanent wilting			Ultimate wilting		
				Soil-moisture tension	Osmotic concentration	Soil-moisture tension + osmotic concentration	Soil-moisture tension	Osmotic concentration	Soil-moisture tension + osmotic concentration
		Percent	*Atmos.*	*Atmos.*	*Atmos.*	*Atmos.*	*Atmos.*	*Atmos.*	*Atmos.*
49	65.3	0.06	0.37	8.8	4.2	13.0	23.7	6.0	29.7
74	61.2	.06	.34	8.0	3.4	11.4	35.5	6.3	41.8
79	58.7	.04	.26	7.0	2.2	9.2	28.9	4.0	32.9
73	74.2	.08	.31	9.2	3.3	12.5	27.0	5.5	32.5
33	45.8	.04	.33	7.7	1.9	9.6	18.4	2.4	20.8
38	53.7	.10	.71	5.0	4.6	9.6	16.4	6.7	23.1
80	76.0	.05	.30	7.8	2.6	10.4	27.0	4.3	31.3
72	89.3	.08	.35	8.8	3.5	12.3	20.5	5.5	26.0
15	62.0	.36	1.47	5.9	9.8	15.7	20.9	14.5	35.4
14	66.7	.06	.22	8.7	1.5	10.2	16.0	1.7	17.7
22	71.9	.07	.36	9.7	2.4	12.1	21.4	2.9	24.3
78	83.7	.06	.31	5.8	2.3	8.1	24.8	4.5	29.3
18	70.7	.11	.76	7.2	4.6	11.8	21.9	6.6	28.5
16	85.6	.07	.33	7.2	2.3	9.5	24.0	2.7	26.7
8	72.5	.06	.34	12.9	1.9	14.8	42.2	2.4	44.6
25	82.9	.07	.37	7.0	2.1	9.1	25.2	2.5	27.7
23	88.7	.26	.91	6.3	5.6	11.9	18.4	7.8	26.2
1	88.3	.16	.73	8.2	3.9	12.1	23.2	4.9	28.1
10	79.4	.04	.21	13.2	1.0	14.2	27.6	1.2	28.8
3	87.9	.08	.37	11.2	1.8	13.0	27.6	2.2	29.8
20	102.8	.11	.40	8.9	2.0	10.9	21.9	2.4	24.3
7	92.4	.08	.33	7.2	1.4	8.6	58.2	1.8	60.0
24	114.0	.13	.40	5.6	2.0	7.6	23.7	3.0	26.7
6	126.2	[1] 1.60	3.77	7.5	16.0	23.5	24.0	20.1	44.1

[1] This soil was leached before determining the wilting percentages.

The frequency diagrams in figure 7 summarize the moisture-retention data at the two wilting values. It is seen that first permanent wilting for these soils occurred in the tension range 5 to 13 atmospheres, with 14 out of the 24 soils wilting in the 7- to 9-atmosphere range. When osmotic effects are added to soil-moisture tension it is seen that at first permanent wilting these soils are distributed fairly uniformly over the equivalent pressure range from 7.5 to 16 atmospheres.

---

[10] MAGISTAD, O. C., AYERS, A. D., WADLEIGH, C. H., and GAUCH, H. G. EFFECT OF SALT CONCENTRATION, KIND OF SALT, AND CLIMATE ON PLANT GROWTH IN SAND CULTURES. Plant Physiol. 18: 151–166. 1944.

[11] This calculation is only an approximation, since it involves the assumption that the total dissolved solids and the degree of ionization remain unchanged as the plants dry the soil from the initial extractions percentage to the wilting points. Also the factor 28.5 is not constant but depends on the composition of the salts present.

Ultimate wilting occurs over a much wider tension range than first permanent wilting. The soil-moisture tension at ultimate wilting was below 30 atmospheres for all but 3 of the soils, and 17 out of the 24 soils underwent permanent wilting in the tension range from 20 to 30 atmospheres. Combining osmotic pressure with soil-moisture tension at ultimate wilting causes no significant rearrangement or grouping of the points in the frequency diagram.

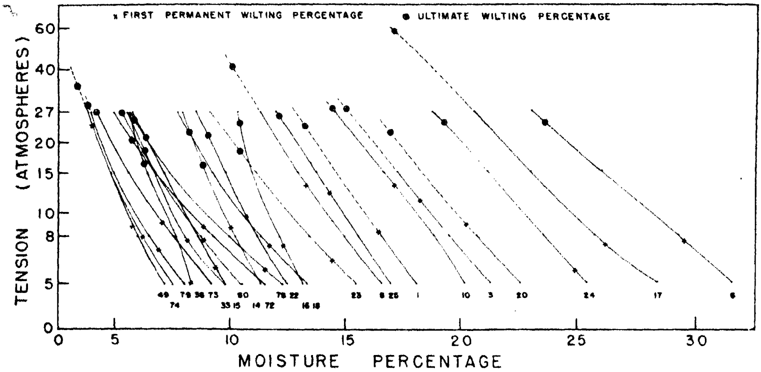


Figure 6.—Moisture-retention curves in the wilting range as determined with pressure-membrane apparatus. The ultimate and first permanent wilting percentage points provide an indirect indication of the range in tension.

One conclusion that might be drawn from figure 7 is that the phenomena of first permanent and ultimate wilting occur over a range in tension or free energy. Unfortunately these results must be regarded as tentative, because the moisture-retention curves in figure 6 were determined when many of the soil samples were nearly exhausted from subsampling by different people, and it is possible that the final samples were not entirely representative of the original samples in which the sunflowers were grown.

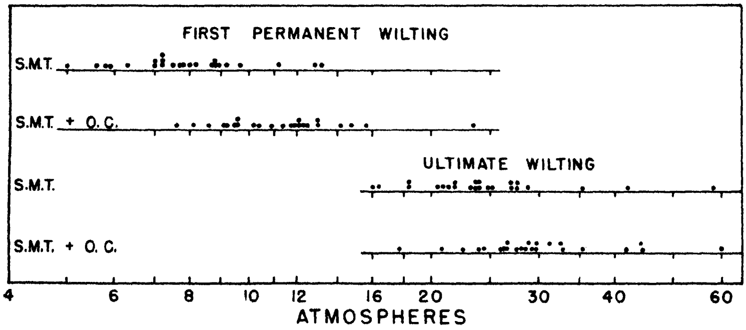


Figure 7.—Soil-moisture tension and sum of soil-moisture tension and osmotic concentration at first permanent wilting and ultimate wilting.

MOISTURE MOVEMENT IN THE WILTING RANGE

The pressure-membrane apparatus appears to provide a useful means for studying moisture movement in relatively dry soils. The curves in figure 8 show typical summation extraction data from which the curves in figure 6 were determined. The zero of the water-extracted scale was taken at the 5-atmosphere equilibrium, and to conserve space in graphing the curves were returned to the zero of the time scale at each pressure increment. The extraction pressures are indicated on the curves.

A burette clamp was used to mount an ordinary 100-ml. stopcock burette on one of the tripod legs of the extraction cell, and the extracted solution was led to the burette tip through a 0.16-mm.-bore copper tube which was closely coupled with rubber tubing. With this arrangement, the gas diffusing through the membrane keeps the extracted solution transported to the burette and a solution outflow of 0.10 ml. or less is easily detectable. The cross on each curve indicates the burette reading at which equilibrium was attained and beyond which no further outflow took place. It is significant that when the 15-atmosphere equilibrium was attained no further outflow took place during a 10- to 15-hour period, but when the extraction pressure was stepped up to 27 atmospheres outflow immediately commenced and continued until a new equilibrium was reached.

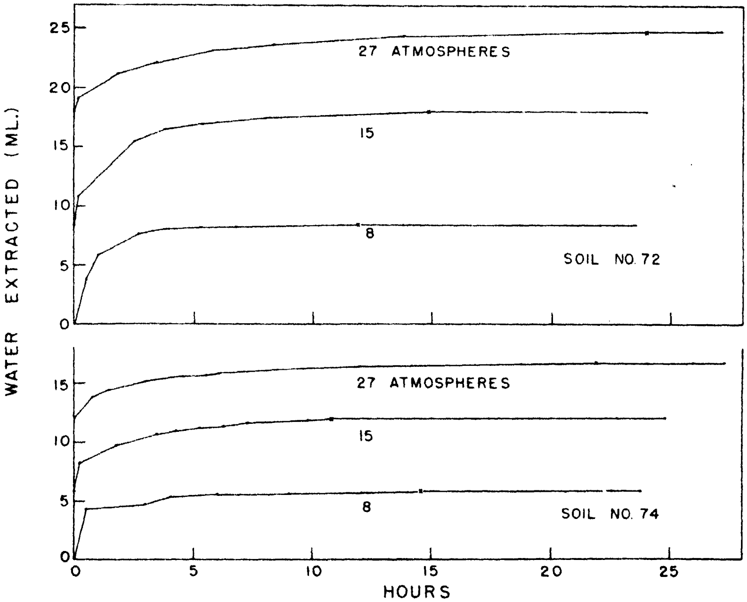


FIGURE 8.—Summation extraction data for two soils, indicating rate of water movement in the 5- to 27-atmosphere tension range. The data were obtained from 400 gm. of soil on a Visking membrane having an area of 660 cm.^{2}

There is no indication that 27 atmospheres is anywhere near the limit for this type of experiment either for the satisfactory operation of the membrane or the moisture-transmitting properties of the soil. Moisture extraction through a Visking membrane has been continued at 15 atmospheres for periods as long as a month with no apparent weakening of the membrane or development of leaks. There is a steady diffusion of gas through the membrane during extraction, and this diffusion rate for nitrogen is approximately 1.3×10^{-6} ml. cm.^{-2} sec.^{-1} atmos.^{-1}. Apparently iron rust has a decidedly deteriorating effect on the membrane and will cause leaks in a short time. Trouble from this source can be prevented by a protective coating on the cylinder of the extraction cell.

On the basis of experiments by Lewis (*10*), it is inferred that moisture movement of the type illustrated in figure 8 is over the surface of the soil and that vapor transfer plays a minor role in the absence of temperature gradients.

The statement is repeatedly made in the literature that moisture movement in unsaturated soil ceases at some moisture content not far below the field capacity. This is substantially true for practical purposes when dealing with such problems as the motion of a 12-inch irrigation into a 6-foot layer of dry soil, but one would hesitate to say that moisture movement of the magnitude shown by the 27-atmosphere curves in figure 8 is of no practical importance in time of drought to plants with established root systems.

SOIL-MOISTURE ENERGY RELATIONS

Various means have been used in the past [12] (*4*, *17*) for expressing the energy of retention of water by soil, or the physical condition of water in soil at various moisture contents. The work involved per unit mass in the transfer of a small element of water between a reference state such as a free flat water surface and the moisture system in soil can be expressed in terms of the thermodynamic function partial molal or partial specific free energy. Edlefsen (*7*) and Edlefsen and Anderson (*8*) have recently discussed this function and its usefulness in connection with soil moisture and plant work. Various physical processes and mechanisms contribute to the retention of water by soil, but the free-energy function seems to be suitable for the most general treatment of soil-moisture problems from the energy standpoint. Unfortunately, convenient and accurate methods for measuring the free energy of soil moisture over the plant-growth moisture range are not now available. Vapor-pressure methods do not yet have sufficient precision. Free-energy determinations from freezing-point depression measurements have been made with some success by Schofield and Botelho da Costa (*18*), Bodman and Day (*1*), and Edlefsen and Anderson (*8*), but results appear to depend on the experimental procedure used and difficulties are encountered at moisture contents in the wilting range. As improvements in measuring methods are made (*19*), it is likely that correct use of the theory in calculating the free energy of soil water from freezing-point data will become easier.

On the basis of experimental results obtainable with pressure-membrane apparatus, it is convenient to divide the forces contributing to the energy of retention of moisture by soil into two classes: (1)

---

[12] DAY, P. R. THE MOISTURE POTENTIAL OF SOILS BY THE CRYOSCOPIC METHOD. 132 pp. 1940. [Thesis on file at Univ. Calif., Berkeley, Calif.]

Those arising from dissolved materials as expressed in terms of osmotic concentration of an extracted sample of the soil solution and (2) all other forces. Force action of the second class can be measured by the use of membranes permeable to the soil solution. The physical quality that is determined experimentally by such membranes is the negative pressure to which a solution must be subjected to be at equilibrium through the membrane with the same solution in the soil.

Past discussions of soil-moisture energy relations have often been confused or ambiguous in their handling of osmotic effects. In spite of its historical significance, the usefulness of capillary potential is considerably lessened by its indefiniteness and by the fact that it is sometimes used as including and sometimes as excluding osmotic effects. It is clear that soil-moisture retention data obtained with tensiometer, suction-plate, pressure-membrane, or centrifugation apparatus are independent of and do not involve solution concentration effects except insofar as the presence of soluble material changes such physical properties of the system as surface tension and density of the soil solution or hydration and flocculation of the soil colloid. Schofield (*17*), in a fruitful and stimulating paper, proposed the pF as a free-energy scale, specifying vapor-pressure and freezing-point methods for its determination. But in the same paper he expressed suction-plate and centrifugation data in terms of pF, thereby neglecting without comment the effect of soluble salts on pF. Many other writers have perpetuated this error in the literature (*9*, *15*, *21*).

If pF is to be accepted as a free-energy scale it should be correctly used and should be clearly distinguished from pressure deficiency or soil-moisture tension. In leached soils, of course, the osmotic component of the pF can be negligible, but in normal soils from semiarid or irrigated regions, dissolved material may account for the major part of the free energy of the soil water. For example, Botelho da Costa (*2*) measured freezing points for 14 California soils supplied by Veihmeyer and found that the average pF at the moisture equivalent was 3.07. Day, [13] using a different freezing-point technique on another set of 14 California soils, found the average value of the pF at the moisture equivalent to be 2.97. If osmotic effects are disregarded, the calculation of tension values from these pF values gives 1,175 and 987 cm. of water, whereas both theoretical and experimental results in a preceding section of this paper indicate that the soil-moisture tension at a moisture percentage equal to the moisture equivalent will account for less than half of these energy values.

At this laboratory, where the effects of salt on the growth and yield of plants are being studied, attempts are being made to segregate and evaluate the effects of soil-moisture tension and osmotic concentration as they operate to determine the availability of moisture to plants. Apparently, considerable work must be done before the energetics of wilting will be well understood, because at present, information on salt effects related to this phenomenon are fragmentary and conflicting.

There is a simple but significant experiment that seems to have a direct bearing on the relation of salt to moisture movement in soil. If a tensiometer is filled with distilled water and the manometer is allowed to attain an equilibrium reading with the porous cup standing in distilled water at a fixed level above the porous surface, it is found that the manometer indicates practically no change in pressure (less than 0.003 atmosphere) when saturated sodium chloride or other strong salt solution is substituted for the distilled water surrounding the cup. From this it is inferred that in soils in the absence of semipermeable membranes, moisture flow is produced primarily by gravity and gradients in soil-moisture tension and not directly by solution concentration gradients. The semipermeable characteristic of the plant root with its discriminating action against the uptake of most of the common salts must cause a build-up of the salt concentration at the root surface during moisture absorption, and it is possible that a correct appraisal of the root environment with regard to osmotic effects may be even more difficult than with respect to soil-moisture tension.

---

[13] See footnote 12, p. 232

SUMMARY

By means of porous ceramic and cellulose membranes, a study of soil-moisture retention has been made on samples of 71 southern California soils for which Furr and Reeves determined the moisture equivalent, the first permanent wilting percentage, and the ultimate wilting percentage.

It was found that for 64 of the 71 soils studied the 15-atmosphere percentage lies in the wilting range somewhere between the first permanent wilting percentage and the ultimate wilting percentage. The soil-moisture tension at first permanent wilting for sunflowers was found to range from 5 to 13 atmospheres, but the majority of the soils showed first permanent wilting in the 7- to 9-atmosphere range. The soil-moisture tension at ultimate wilting was below 30 atmospheres for all but 3 of the soils, and 17 out of the 24 soils tested underwent permanent wilting in the range from 20 to 30 atmospheres. Moisture transfer in soils at moisture contents in the wilting range, as indicated by the rate of extraction of moisture from soil in the pressure-membrane apparatus at 15 and 27 atmospheres, is apparently more rapid than can be accounted for by vapor diffusion and should be of practical importance to plant-root systems under drought conditions.

The moisture equivalent is the average value over approximately the 0.1- to 1.0-atmosphere tension range for a moisture retention curve that takes into account centrifuge packing effects. From determinations made on a suction plate it was found that, on an average for the 71 soils studied, the moisture retained by an air-dried and screened but uncentrifuged sample at a tension of one-third of an atmosphere corresponds closely to the moisture equivalent. A set of moisture-retention curves, covering the tension range from 2 to 20,000 cm. of water and for a wide range of soil textures, shows considerable intercrossing of the various curves throughout the whole tension range.

Tensiometer, suction-plate, pressure-plate, pressure-membrane, or centrifugation apparatus may be used for determining equivalent negative pressure or soil-moisture tension, but, without disregarding osmotic effects, none of these can be used for determining pF if the latter is to be taken as a free-energy scale as originally proposed.

LITERATURE CITED

(1) Bodman, G. B., and Day, P. R.
1937. THERMOELECTRIC METHOD OF DETERMINING THE FREEZING POINTS OF SOILS. Soil Sci. Soc. Amer. Proc. 2: 65–71, illus.

(2) BOTELHO DA COSTA, J. V.
1938. THE INDIRECT DETERMINATION OF THE "WILTING COEFFICIENT" BY THE FREEZING-POINT METHOD, AND THE INFLUENCE OF THE SALTS UPON pF AT THAT CRITICAL MOISTURE CONTENT. Jour. Agr. Sci. [England] 28: 654–662, illus.

(3) BROWNING, G. M.
1941. RELATION OF FIELD CAPACITY TO MOISTURE EQUIVALENT IN SOILS OF WEST VIRGINIA. Soil Sci. 52: 445–450.

(4) BUCKINGHAM, E.
1907. STUDIES ON THE MOVEMENT OF SOIL MOISTURE. U. S. Dept. Agr., Bur. Soils Bul. 38, 61 pp., illus.

(5) CHILDS, E. C.
1942. STABILITY OF CLAY SOILS. Soil Sci. 53: 79–92, illus.

(6) EATON, F. M., and HORTON, C. R.
1940. EFFECT OF EXCHANGE SODIUM ON THE MOISTURE EQUIVALENT AND THE WILTING COEFFICIENT OF SOILS. Jour. Agr. Res. 61: 401–425, illus.

(7) EDLEFSEN, N. E.
1941. SOME THERMODYNAMIC ASPECTS OF THE USE OF SOIL-MOISTURE BY PLANTS. Amer. Geophys. Union, Trans. 1941 (3): 917–926.

(8) ——— and ANDERSON, A. B. C.
1943. THERMODYNAMICS OF SOIL MOISTURE. Hilgardia 15: 31–298, illus.

(9) JAMISON, V. C.
1942. STRUCTURE OF SOME ORGANIC SOILS AND SOIL MIXTURES AS SHOWN BY MEANS OF pF MOISTURE STUDIES. Amer. Soc. Agron. Jour. 34: 393–404, illus.

(10) LEWIS, M. R.
1937. RATE OF FLOW OF CAPILLARY MOISTURE. U. S. Dept. Agr. Tech. Bul. 579, 29 pp., illus.

(11) OLMSTEAD, L. B.
1937. SOME MOISTURE RELATIONS OF THE SOILS FROM THE EROSION EXPERIMENT STATIONS. U. S. Dept. Agr. Tech. Bul. 562, 45 pp., illus.

(12) RICHARDS, L. A.
1941. A PRESSURE-MEMBRANE EXTRACTION APPARATUS FOR SOIL SOLUTION. Soil. Sci. 51: 377–386, illus.

(13) ———
1942. SOIL MOISTURE TENSIOMETER MATERIALS AND CONSTRUCTION. Soil Sci. 53: 241–248, illus.

(14) RUSSELL, M. B.
1939. SOIL MOISTURE SORPTION CURVES FOR FOUR IOWA SOILS. Soil Sci. Soc. Amer. Proc. 4: 51–54, illus.

(15) ——— and RICHARDS, L. A.
1938. THE DETERMINATION OF SOIL MOISTURE ENERGY RELATIONS BY CENTRIFUGATION. Soil Sci. Soc. Amer. Proc. 3: 65–69, illus.

(16) SCHAFFER, R. J., WALLACE, J., and GARWOOD, F.
1937. THE CENTRIFUGE METHOD OF INVESTIGATING THE VARIATION OF HYDROSTATIC PRESSURE WITH WATER CONTENT IN POROUS MATERIALS. Faraday Soc. Trans. 33: 723–734, illus.

(17) SCHOFIELD, R. K.
1935. THE pF OF WATER IN SOIL. Internatl. Cong. Soil Sci. Trans. 3 (2): 37–48, illus.

(18) ——— and BOTELHO DA COSTA, J. V.
1938. THE MEASUREMENT OF pF IN SOIL BY FREEZING POINT. Jour. Agr. Sci. [England] 28: 644–653, illus.

(19) SHAW, T. M.
1942. METHOD OF MINIMIZING SUPERCOOLING AND ITS APPLICATION IN THE DETERMINATION OF FREEZING POINTS FROM DIELECTRIC CONSTANT MEASUREMENTS. Rev. Sci. Instruments 13: 2–5.

(20) VEIHMEYER, F. J., and HENDRICKSON, A. H.
1928. SOIL MOISTURE AT PERMANENT WILTING OF PLANTS. Plant Physiol. 3: 355–357.

(21) WOODRUFF, C. M.
1940. SOIL MOISTURE AND PLANT GROWTH IN RELATION TO pF. Soil Sci. Soc. Amer. Proc. 5: 36–41, illus.

DEFICIENCY, TOXICITY, AND ACCUMULATION OF BORON IN PLANTS[1]

By FRANK M. EATON[2] [3]

Formerly *plant physiologist, Division of Irrigation Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In some irrigated areas of the West, the concentration of boron in the water supply is so high as to cause injury to field and dooryard plantings, whereas in other areas the concentration is so low as to suggest that here and there boron applications might improve the growth of plants with high boron requirements.

The symptoms of boron deficiency and boron excess are often so striking in character that they are of great value in the interpretation of the boron conditions existing on irrigated lands as well as on agricultural lands generally. The symptoms of deficiency and injury show so much variation, however, between species in respect both to character and intensity, that background information of definite nature is almost a requisite to satisfactory diagnosis or reliable conclusions.

The work on which this paper is based was begun in 1929. Since the work was completed, much has been published on the morphological changes and physiological reactions induced by a lack or an excess of boron, together with other results on many of the plants included herein. Inasmuch as all or nearly all of this literature has found a place in comprehensive abstracts, it has not seemed necessary to review it here.

The present paper reports the results of tests to determine the symptoms, growth reactions, and boron-accumulation characteristics of plants grown in sand cultures supplied with different quantities of boron.

MATERIAL AND METHODS

MATERIAL

Fifty species of plants (58 varieties) were grown out of doors in each of 6 large sand cultures. These cultures were supplied with nutrient solutions containing a trace (0.03 to 0.04 p. p. m.), 1, 5, 10, 15, and 25 parts per million of boron, respectively. When the tolerance

---

[1] Received for publication December 24, 1942.

[2] Now senior plant physiologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[3] The writer expresses his thanks to Geo. Y. Blair, for aid in the care of plants and samples; to L. V. Wilcox and J. H. Hatcher, for boron analyses; to M. S. Mayhugh, for photographs; and to V. P. Sokoloff, for analyses of substances other than boron.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 6
Sept. 15, 1944
Key No. G-1300

of the plants and other conditions of the experiment permitted, the plantings were carried to an advanced stage of maturity. The weights of the plants produced and the concentrations of boron in the leaves and other parts were determined. The inclusion of so large a number of species in the work limited the number of plants of each that could be grown and, owing to the small populations, it was necessary to select carefully the plants for transplanting or to plant many extra seeds so that offtype, especially vigorous, or undersized individuals could be discarded during the successive stages of thinning.

The investigation was conducted at Riverside, Calif., and the data reported were obtained during the period from May 1930 to March 1934. There were four summer and four winter plantings, each of which included six or more varieties grown together in each of the sand cultures.

PLAN OF PROCEDURE

The investigation was begun on an exploratory basis in 1929 with five sand beds. The summer and winter plantings of that year provided an opportunity for orientation not only in regard to the diversity and nature of the boron reactions of plants but also for the improvement of experimental procedure. Although the 1929 data are not tabulated in this paper, they are regarded as valid and are occasionally referred to for additional information on crops grown during the succeeding years.

The 1929 results served to emphasize the diversity in the boron requirements and tolerance of different species and indicated that six boron concentrations probably represented the minimum number of treatments that could reasonably be expected to serve the purposes of the investigation. Accordingly, it became necessary to evaluate the advantages, on the one hand, of using fewer concentrations of boron in the interest of replicated treatments and the customary criteria of statistical significance or, on the other hand, of using the six concentrations and relying on departures from the growth and boron-accumulation trends and on the development of plant symptoms through the series of successively higher concentrations as observational indications of plant variability and significance. The latter course was followed.

Substantial diversity was found in the growth and boron-accumulation characteristics of plants included in more than one experiment. Inasmuch as the same methods were followed throughout the work, it has seemed most reasonable to associate such differences in behavior with differences in weather conditions. The weather data presented in table 1 (p. 243) cover the last half of the growth period of each crop and include the average daily maximum temperature, relative humidity at 12 noon, evaporation per week from a standard 4-foot tank, and hours of sunshine, all of which are stated as weekly averages taken to the nearest Monday from the records of the Citrus Experiment Station Riverside, Calif. In its influence on the boron concentrations in the plants at time of harvest, the weather during the final half of the growth period has, beyond reasonable doubt, a greater significance than that of the full period. The early growth rate may often go far in determining ultimate plant size, but it is usually true that more than half of the total carbon assimilation occurs during the final half of the period of growth.

In these experiments growth was always conditioned to some extent by the proximity of the plants to neighboring plants. Excessive exposure was regarded as nontypical of field conditions, and for that reason an effort was made in arranging and spacing the plantings to anticipate the magnitude of the subsequent growth so that neither shading nor exposure would be excessive; unavoidably there were departures from the conditions regarded as ideal. Because of their curtailed growth the high-boron plants, in nearly all instances, were more exposed to light and wind than the low-boron plants; but this effect, while probably altering some of the plant reactions, was not regarded as especially undesirable from an agronomic viewpoint, inasmuch as similar relations exist in field plantings. The spacing and order of planting were always the same in all of the treatments of any experiment.

The base nutrient employed proved to be well suited to the purpose of the experiment and supported a creditable growth of all plants, but it does not follow that it was the most favorable solution possible for any one of the plants or that it was equally suitable during the successive stages of plant development. This particular culture solution would not have been satisfactory had the hydrogen-ion concentrations been controlled at a pH value as low as 6, for example, since in such cases higher concentrations of phosphate would have remained in solution and iron chlorosis would have resulted in at least some of the species.

As a rule the boron determinations on the plant material were not run in duplicate; to have done so would have made it necessary to omit many of the analyses of the different plant parts. The method employed for the determination of boron in plant material has customarily been found to be accurate to within 10 or 15 p. p. m., but throughout the data there are indications that errors of greater magnitude sometimes occurred. In the interpretation of the boron measurements, as was indicated in the instance of plant weights, a measure of emphasis can properly be placed on the trends through the successive six treatments.

Boron accumulates in leaves as they age, and leaf abscission in a number of plants is one of the most noticeable effects of excess accumulation. This fact made it necessary to decide in advance whether it would be better to save leaves as they fell from plants, including them in the total weight and in the samples for analyses, or to let the intact portion of the plant and its boron content at time of harvest stand as the criterion of effect of the treatment. The latter course was followed, but the loss of old leaves high in boron unquestionably resulted in many instances in lower concentrations than would have been found if all dead leaves had been saved.

METHODS

The outdoor sand-culture equipment (*5*)[4] is illustrated in figure 1, which shows the crops and sand beds in July 1933. Except as noted in the tables, the seeds were planted directly in the sand and supplied from the start with culture solutions containing the designated concentrations of boron. The sand in these beds retained approximately 140 liters of solution against gravity. The solution retained by the sand was displaced once or twice each day by flooding the surface of the sand beds with 180 liters of solution. The displaced solutions drained into auxiliary reservoirs, where they were made up to volume, by the addition of water, for reuse. As soon as the plants in any experiment had become well established, new solutions (180 liters) were systematically substituted for those in use, at intervals customarily of 5 to 15 days, depending on the amount of vegetation in the beds and the climatic conditions.

---

[4] Italic numbers in parentheses refer to Literature Cited, p. 277.

Throughout the experiment the new base nutrient solution for all treatments was made up with 6, 3, and 3 millimoles per liter, respectively, of calcium nitrate, magnesium sulfate, and monopotassium phosphate. Manganese was added to produce a concentration of 0.5 p. p. m., and iron tartrate was supplied as needed. Traces of zinc were derived from the chemicals in the culture solution and from the galvanized pipes. Distilled water was used for making up and replenishing the culture solutions in the trace-boron bed, and tap water was used for the others.

FIGURE 1.—Sand cultures in July 1933.

Occasionally complete analyses were made of the used solutions. None of these analyses indicated that any of the constituents of the base nutrient were ever sufficiently low to retard growth. An extensive precipitation of calcium phosphate occurred when the new solutions were mixed with the more alkaline residual solutions retained in the sand, and further precipitation occurred as the hydrogen-ion concentrations decreased with the use of solutions. As a consequence of this precipitation, the concentration of HPO_4 ion actually present in the solutions in the sand during the short cycles between solution changes varied from about 0.4 to 0.1 milliequivalent per liter.

Measurements of hydrogen-ion concentrations were made on all new and discarded solutions. The pH values of the new solutions before passage through the sand, with few exceptions, were in the 6.3 to 6.5 range, and those of the used solutions, at the time of discarding, were in the 6.9 to 7.7 range, values between 7.3 and 7.5 being the most typical.

The boron determinations on both plant materials and culture solutions were made by methods described by Wilcox (*14, 15*). The average of 31 boron determinations made on new trace-boron solutions was 0.03 p. p. m., and the average of 40 determinations on solutions as discarded was 0.04 p. p. m. The lowest concentration found was an undeterminable trace and the highest was 0.11 p. p. m. The analyses showed no evidence of significant differences in the boron concentrations of the solutions used for the successive plantings.

In harvesting the plants it was customary to flush the beds with solution and, while the sand was saturated or nearly so, to pull out the roots. These pulled roots are included as a part of the entire plant weight unless otherwise indicated.

EXPERIMENTAL RESULTS

PLANTS INCLUDED IN SURVEY

The data on the growth reactions and the boron-accumulation characteristics of the plants included in the investigation are reported in table 1, in the order of the relative tolerance of the plants to boron. These plants and their position by number in table 1 are as follows:

Alfalfa (47–49), artichoke (71), asparagus (72), barley (20), blackberry (1), cabbage (35), calendula (38), California-poppy (56), carrot (28, 29), celery (44), cherry (4), common beet (58, 59), corn (32, 33), cotton (70), cowpea (16), elm (3), fig (7), grape (10, 11), Jerusalem-artichoke (17), Kentucky bluegrass (31), kidney bean (14, 15), larkspur (18), leaf beet (60), lemon (2), lettuce (50, 51), lima bean (23), lupine (9), milo (36, 37), muskmelon (61), mustard (45), oats (43), onion (25–27), oxalis (69), pansy (13), parsley (46), pea (21, 22), peach (5), persimmon (6), potato (34), radish (39–42), redpepper (30), strawberry (8), sugar beet (65–68), sweetclover (62, 63), sweet pea (64), sweetpotato (24), tobacco (52), tomato (54,55), turnip (57), vetch (53), violet (12), and zinnia (19).

TABLE 1.—*Boron accumulation in various plants and its effect*

BORON-SENSITIVE

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
1	Blackberry (*Rubus* sp.).	Mammoth Thornless.	Apr.	Nov.	1	S	Trace		1	All
2	Lemon (*Citrus limonia* Osbeck).	Eureka, cuttings rooted in December (4 plants).	May	Sept.	1	None	Trace		1	do
3	Elm (*Ulmus americana* L.).	American, selected 12-inch seedlings.	Apr.	Nov.	1	S	1		1	do.
4	Cherry (*Prunus avium* L.).	Mazzard, seedlings from previous year (3 plants).	Apr.	Nov.	1	S	1		5	do
5	Peach (*Prunus persica* (L.) Batsch).	Seedlings from previous year (2 plants).	Apr.	Nov.	2	None	1		5	do
6	Persimmon (*Diospyros kaki* L., f.).	Kaki, seedlings from previous year cut back (3 plants).	Apr.	Nov.	1	S	1		1	do
7	Fig (*Ficus carica* L.).	Kadota, year-old cuttings with tops and roots cut back (2 plants).	Apr.	Nov.	1	S	1		5	do
8	Strawberry (*Fragaria* sp.).	Klondike (3 plants).	Nov.	Mar.	3	None	1		5	do
9	Lupine (*Lupinus hartwegi* Lindl.).	Hartweg (5 plants).	Oct.	Apr.	5	M	1		5	do
10	Grape (*Vitis vinifera* L.).	Malaga, single year-old plant cut back.	Apr.	Nov.	1	S	1	9	5	do
11	do	Sultanina, single year-old plant cut back.	May	Sept.		S	1		5	All (green).
12	Violet (*Viola odorata* L.).	Princess of Wales (4 transplants).	Oct.	Mar.	4	None	Trace	20	5	All
13	Pansy (*Viola tricolor* L.).	5 small transplants.	Oct.	Mar	5	None	Trace	24	5	do

See footnotes at end of table.

on growth, with other symptoms, under different climatic conditions

PLANTS

Dry weight [5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants [6] grown in solution with indicated concentration of boron (p. p. m.)						Average climaitc conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*°F.*	*Pct.*	*In.*	*Hr.*
145	137	10	...	...	...	Leaves	9	210	717	...	...	...	82.9	69.3	1.42	9.1
						Stems	12	18	...	...	...	...				
						Roots	16	68	...	...	...	...				
						Weighted mean.[7]	11	122	...	...	...	...				
47	27	11	...	...	...	Leaves	61	314	1,232	...	...	...	97.3	70.0	1.91	11.0
						Other parts	11	33	54		...	...				
						Weighted mean.	34	147	589	...	...	...				
145	150	27	...	...	...	Leaves	27	277	943		...	...	89.6	68.5	1.45	9.2
						Stems	13	14	22	...	...					
						Roots	18	35	43	...	...					
						Weighted mean.	18	103	197		...	...				
61	63	16	...		...	Leaves	14	104	182	...	...	...	89.6	68.5	1.45	9.2
						Stems	2	23	85	...		...				
						Roots	2	54	...	...	...	...				
						Weighted mean.	6	49	...	...	...	...				
363	396	172			...	Leaves	17	81	170		...	...	89.6	68.5	1.45	9.2
						Stems	7	44	138	...	...	...				
						Roots	2	53	192	...	...	...				
						Weighted mean.	8	57	160	...	...	...				
64	71	36	10	...	...	Leaves	40	389	1,182	...		...	89.9	68.5	1.45	9.2
						Stems	31	22	28	56	...	...				
						Roots	8	46	77	163	...	...				
						Weighted mean.	28	132	362	...	...	...				
169	455	138	10	...	...	Leaves	15	404	722	1,296	...	...	89.6	68.5	1.45	9.2
						Roots	10	28	63	...	...	...				
11	12	4	4	3	...	All	44	113	315	450	755	...	75.8	67.1	.79	6.2
57	79	11	4	...	...	do	27	96	367	815	...	...	68.6	66.0	.91	7.5
150	379	74	18	15	...	Leaves	38	250	926	1,774	...	...	89.2	69.3	1.42	9.1
						Stems	28	50	50	155	...	...				
383	720	145	125	...	...	Leaves	86	267	1,804	...	...	...	97.3	70.0	1.91	11.0
35	34	14	5	2	...	All	13	49	153	274	262	...	64.0	69.8	.73	6.9
75	47	33	17	4	...	do	16	59	275	504	822	...	74.8	62.5	.78	6.6

TABLE 1.—*Boron accumulation in various plants and its effect on growth,*

BORON-SENSITIVE

Crop										
No.	Name	Variety and description	Date planted	Date cropped	Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
14	Kidney bean (*Phaseolus vulgaris* L.).	Navy (5 plants)	May	Sept.	5	None	1	..	1	All
15	do	Navy (8 plants)	June	Aug.	8	D	Trace	32	1	do
16	Cowpea (*Vigna sinensis* (Torner) Savi).	Whippoorwill (8 plants).	June	Aug.	8	D	Trace	30	5	do
17	Jerusalem-artichoke (*Helianthus tuberosus* L.).	From tubers (8 plants).	Apr.	July	8	None	1	34	1	do
18	Larkspur (*Delphinium* sp.).		Oct.	Mar.	11	S	1	43	5	do
19	Zinnia (*Zinnia elegans* Jacq.).	Yellow (7 plants)	May	Sept.	7	None	Trace	45	1	do
										BORON-SEMI-
20	Barley (*Hordeum vulgare* L.).	California Common Coast, cropped when partly ripe (21 plants).	Apr.	July	21	D	Trace	51	5	Grain Other Total
21	Pea (*Pisum sativum* L.).	Hundredfold (5 plants).	Dec.	May	5	None	1	55	5	All
22	do	American Wonder (5 plants).	Oct.	Mar.	5	..	1	53	1	do
23	Lima bean (*Phaseolus lunatus* L.).	Burpee Bush (5 plants).	May	Sept.	5	S	Trace	57	1	do
24	Sweetpotato (*Ipomoea batatas* (L.) Lam.).	(2 plants)	June	Oct.	2	S	Trace	63	5	Tops Roots Total
25	Onion (*Allium cepa* L.).	Riverside Sweet Spanish (7 plants in 10-boron, 12 elsewhere).	Dec.	Apr.	12	None	Trace	68	1	Leaves Roots Total
26	do	Riverside Sweet Spanish (6 plants cropped early).	May	July	6	None	Trace	75	1	Leaves Roots Total

See footnotes at end of table.

with other symptoms, under different climatic conditions—Continued

PLANTS—Continued

Dry weight[5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants[6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	°*F.*	*Pct.*	*In.*	*Hr.*
322	401	210	5	...	...	All	12	191	648	645	...	...	96.5	68.5	2.03	10.1
628	583	376	189	39	...	Leaves	Trace	151	690	1,254	1,733	...	96.6	66.0	1.94	11.5
						Other	11	27	41	89	138	...				
						Weighted mean.	6	85	359	705	997	...				
422	332	259	101	19		Leaves	32	119	404	722	1,132	...	96.6	66.0	1.94	11.5
						Other	22	30	59	99	167	...				
						Weighted mean.	27	70	195	296	573					
628	640	372	189	93	...	Dead leaves	171	519	986	1,550	1,590	...	87.4	69.0	1.28	6.7
						Other	27	92	221	353	427					
						Weighted mean.	28	98	377	746	1,001	...				
110	168	114	75	29	14	All	124	110	286	602	918	820	73.9	62.2	.73	6.5
693	648	439	330	158	19	Leaves	90	436	1,241	2,191	2,739	3,080	98.1	68.3	2.05	11.2
						Other	41	76	95	220	237	429				
						Weighted mean.	54	183	424	859	1,187	1,685				
TOLERANT PLANTS																
102	90	65	64	21	19	Grain	3	11	12	27	45	51	88.7	69.0	1.44	7.9
283	238	169	154	112	71	Other	19	104	299	652	953	1,395				
385	328	234	218	133	90	Weighted mean.	15	78	219	469	810	1,111				
195	203	121	105	109	64	Leaves	16	212	609		1,283	1,452	72.5	66.8	.91	8.0
						All	16	64	230	338	436	562				
31	36	26	24	8	5	do	56	110	319	364	435	867	61.7	71.7	.81	6.8
154	...	100	92	70	1	do	26	206	515	1,181	1,298	...	94.3	70.3	1.91	9.9
815	722	698	553	335	73	Tops	16	118	310	602	894	1,410	91.4	69.7	1.21	9.0
358	262	276	278	80	24	Roots	6	44	20	33	88	90				
1,173	984	974	831	415	97	Weighted mean.	13	98	228	412	739	1,083				
21	15	16	13	17	15	Leaves	30	104	270	520	987	1,578	78.0	70.3	1.28	9.5
12	8	8	5	8	7	Roots	23	36	105	150	325	346				
33	23	24	18	25	22	Weighted mean.	27	80	215	417	775	1,186				
9	7	5	7	7	6	Leaves	29	115	354	530	876	1,488	95.0	66.3	2.04	12.2
7	6	5	6	6	3	Roots	...	21	54	64	84	119				
16	13	10	13	13	9	Weighted mean.	...	72	204	315	510	1,032				

TABLE 1.—*Boron accumulation in various plants and its effect on growth,*

BORON-SEMI-

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
27	Onion (*Allium cepa* L.).	Riverside Sweet Spanish (final 6 plants of crop No. 26).	May	Sept.	6	None	Trace	64	1	Leaves
										Roots
										Total
28	Carrot (*Daucus carota* L.).	Danvers Half Long (14 plants).	Dec.	Apr.	14	None	Trace	70	10	Laminae
										Roots
										Other
										Total
29	do	do	May	Sept.	14	None	Trace	70	5	Laminae
										Roots
										Other
										Total
30	Redpepper (*Capsicum frutescens* L.).	Sweet Chinese Giant (3 plants).	Apr.	Oct.	3	None	Trace	71	5	Leaves
										Fruit
										Other
										Total
31	Kentucky bluegrass (*Poa pratensis* L.).	2 18-inch rows cut in May, cropped in June.	Dec.	May	..	None	1	73	1	Leaves
				June	..	None	5	86	1	All
32	Corn (*Zea mays* L.)	Iowa Yellow Dent (3 plants), cropped before flowering.	Apr.	June	3	None	1	53	5	All
33	do	Iowa Yellow Dent (3 plants).	June	Oct.	3	M	5	79	5	Leaves
										Grain
										Other
										Total
34	Potato (*Solanum tuberosum* L.).	British Queen (3 plants; in 10-boron, 4 elsewhere).	Dec.	May	4	S	1	78	1	Leaves
										Tubers
										Other
										Total
35	Cabbage (*Brassica oleracea* var. *capitata* L.).	Early Flat Dutch (3 plants), cropped soon after heading had started.	Dec.	Apr.	3	None	1	78	10	Green laminae.
										Covered laminae.
										Other
										Total
36	Milo (*Sorghum vulgare* Pers.).	Dwarf Yellow (3 plants).	May	Nov.	3	M	Trace	([8])	5	Leaves
										Heads
										Roots
										Other
										Total

See footnotes at end of table.

with other symptoms, under different climatic conditions—Continued

TOLERANT PLANTS—Continued

Dry weight [5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants [6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*°F.*	*Pct.*	*In.*	*Hr.*
16	8	8	8	8	9	Leaves	44	91	242	510	679	926	98.1	68.3	2.05	11.2
37	23	26	27	24	27	Roots	28	46	46	74	87	155				
53	31	34	35	32	36	Weighted mean.	33	58	92	174	235	348				
33	23	30	22	13	12	Laminae	36	66	124	324	542	788	78.0	70.3	1.28	9.5
59	42	56	32	24	14	Roots	22	33	64	154	231	319				
35	34	43	27	18	15	Other	28	55	55	121	148	174				
127	99	129	81	55	41	Weighted mean.	27	48	75	189	277	403				
53	50	45	37	33	30	Laminae	38	204	395	999	1,165	1,244	97.3	70.0	1.91	11.0
26	24	15	14	13	19	Roots	25	57	65	131	117	114				
55	59	51	37	37	32	Other	38	65	103	181	166	209				
134	133	111	88	83	81	Weighted mean.	35	116	216	517	556	570				
119	107	147	81	63	49	Leaves	34	118	328	700	729	882	92.2	71.0	1.40	9.4
224	218	132	171	109	34	Fruit		21	16	24	34	46				
124	101	150	85	55	53	Other		19	24	31	51	48				
467	426	429	337	227	136	Weighted mean.		45	126	188	231	348				
46	49	45	44	20	13	Leaves	3	22	92	164	224	398	72.5	66.8	.91	8.0
18	21	22	20	11	10	All	8	41	153	361	477	1,185	81.8	64.4	1.52	9.5
422	447	337	245	134	48	do	11	32	123	235	377	727	82.5	70.3	1.41	7.5
186	254	183	169	146	120	Leaves	27	72	179				90.0	73.5	1.05	8.4
390	364	699	417	360	229											
633	734	507	372	368	343											
1.209	1,352	1,389	958	874	692											
139	131	111	145	78	57	Leaves	16	98	399	685	1,311	1.644	72.4	64.8	1.08	8.2
591	796	713	599	490	177	Tubers	8	22	13	41	27	65				
102	92	75	118	55	42	Other	22	32	43	75	99	127				
832	1,019	899	862	623	276	Weighted mean.	11	33	63	154	194	401				
140	234	147	172	174	132	Green laminae	16	104	204	440	561	1,152	78.0	70.3	1.28	9.5
23	17	25	22	24	9	Covered laminae.	22	52	55	60	94	110				
108	163	134	138	127	98	Other	28	52		74	116	176				
271	414	306	332	325	239	Weighted mean.	21	81		263	353	713				
285	131	92	117	108	45	Leaves	16	138	625	1,204	1,461	2,009	88.3	68.0	1.32	9.1
678	492	461	378	275	41	Stalks + sheaths.	11	11	23	61	103	261				
253	61	68	79	49	24											
670	206	235	271	200	71											
1,886	890	856	845	632	181											

TABLE 1.—*Boron accumulation in various plants and its effect on growth,*

BORON-SEMI-

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
37	Milo (*Sorghum vulgare* Pers.).	Dwarf Yellow (4 plants).	July	Dec.	4	M	1	80	1	Leaves
										Grain
										Other
										Total
38	Calendula (*Calendula officinalis* L.).	Orange King (7 plants).	Dec.	Apr.	7	None	Trace	80	5	Leaves
										Other
										Total
39	Radish (*Raphanus sativus* L.).	Early Scarlet (15 plants).	Dec.	Feb.	15	S	1	60	10	Leaves
										Roots
										Total
40	do	Early Breakfast (10 plants).	Oct.	Nov.	10	S	Trace	85	10	Leaves
										Roots
										Total
41	do	Early Scarlet (15 plants).	Dec.	Mar.	15	S	5	90	10	Leaves
										Roots
										Total
42	do	Early Scarlet (20 plants).	June	July	20	None	5	91	10	Leaves
										Roots
										Total
43	Oats (*Avena sativa* L.).	Kanota badly lodged and cropped before ripe (21 plants).	Apr.	July	21	None	5	86	5	Grain
										Other
										Total
44	Celery (*Apium graveolens* L.).	Seeded in Aug. and transplanted to cultures (5 plants).	Nov.	Mar.	5	S	15	89	25	Leaflets
										Other
										Total
45	Mustard (*Brassica* sp.).	Yellow-seeded (5 plants).	Oct.	Jan.	5	None	1	94	10	All
46	Parsley (*Petroselinum crispum* (Mill.) Nym.).	Double Curled (12 plants).	Oct.	Mar.	12	None	5	95	15	do
47	Alfalfa (*Medicago sativa* L.).	Chilean (20 plants were left in each bed when thinned; of these 20, 20, 20, 19, 18, and 11 survived respectively).	Dec.	Apr.	....	None	10	106	15	Leaves
										Other
										Total

See footnotes at end of table.

with other symptoms, under different climatic conditions—Continued

TOLERANT PLANTS—Continued

Dry weight [5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants [6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*°F.*	*Pct.*	*In.*	*Hr.*
407	559	409	320	302	201	Leaves + sheaths.	8	24	111	222	292	342	76.8	59.5	.80	7.0
542	543	534	505	292	114	Grain	Trace	Trace	Trace	3	7	13				
890	867	996	719	644	371	Other	Trace	Trace	22	49	70	160				
1,839	1,969	1,939	1,544	1,238	686	Weighted mean.	2	7	35	70	109	189				
70	64	64	46	50	50	Leaves	33	272	715	1,490	1,986	2,106	77.1	71.4	1.24	9.5
123	125	109	100	97	68	Other	11	44	94	110	170	231				
193	189	173	146	147	118	Weighted mean.	19	121	324	545	788	1.025				
19	24	19	14	10	12	Leaves	33	36	58	86	145	209	67.9	75.6	1.03	6.4
9	14	11	7	7	7	Roots	15	20	33	62	58	98				
28	38	30	21	17	19	Weighted mean.	27	30	49	78	109	168				
21	16	19	18	17	10	Leaves	13	55	96	192	197	375	79.0	67.8	.74	7.8
18	16	16	15	14	9	Roots	33	30	49	66	66	116				
39	32	35	33	31	19	Weighted mean.	22	43	75	135	138	254				
33	36	38	39	34	33	Leaves	8	59	155	337	423	714	68.7	64.5	.93	8.0
23	32	31	21	21	13	Roots	24	27	51	99	124	170				
56	68	69	60	55	46	Weighted mean.	15	44	108	254	309	560				
24	28	30	30	21	19	Leaves	24	89	196	407	679	1,151	91.4	69.6	1.44	12.1
20	26	25	22	19	15	Roots	13	30	50	86	127	183				
44	54	55	52	40	34	Weighted mean.	19	61	134	271	417	724				
45	33	64	35	23	9	Grain	13	19	43	107	184	419	88.7	69.0	1.44	7.9
234	280	303	217	167	86	Other	5	69	334	706	1,096	1,748				
279	313	367	252	190	95	Weighted mean.	6	64	283	623	986	1,622				
43	99	80	78	97	54	Leaflets	20	68	140	297	432	720	74.8	67.3	.73	6.0
54	131	94	111	152	99	Other	30	60	118	256	379	731				
97	230	174	189	249	153	Weighted mean.	26	63	128	273	400	729				
443	527	526	460	497	348	All	49	65	80	108	148	205	60.7	69.3	.59	6.0
176	183	214	200	104	3	do	16	43	172	388	588	890	74.8	62.5	.78	6.6
79	70	73	85	77	42	Leaves	28	182	330	627	740	996	78.0	70.3	1.28	9.5
197	209	221	237	195	102	Other	11	14	28	55	52	....				
276	279	294	322	272	144	Weighted mean.	16	56	103	206	247	....				

TABLE 1.—*Boron accumulation in various plants and its effect on growth,*

BORON-SEMI-

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
48..	Alfalfa (*Medicago sativa* L.).	Chilean (20 plants; many ripened seed when cut).	May	July	20	None	15	98	15	Leaves Stems Total
49..	do	Chilean, final cropping; 16, 17, 19, 13, 12, and 10 plants survived in respective beds.		Sept.		Mild	5	75	15	Leaves Stems Roots Total
50..	Lettuce (*Lactuca sativa* L.).	Los Angeles Market (4 plants).	June	Aug.	4	None	5	96	1	Leaves Other Total
51..	do	Big Boston Head (4 plants).	June	Aug.	4	None	5	92	1	Leaves Other Total
52..	Tobacco (*Nicotiana tomentosa* Ruiz and Pav.).	Giant Havana Acclimated (3 plants).	May	Aug.	3	None	15	96	10	Laminae Other Total
53..	Vetch (*Vicia atropurpurea* Desf.).	Purple (12 plants)	Oct.	Jan.	12	None	5	98	5	All
54..	Tomato (*Lycopersicon esculentum* Mill.).	Marglobe, cropped during early fruiting stage (3 plants).	May	July	3	None	10	99	5	Leaves Fruit Other Total
55..	do	Stone, cropped early (3 plants).	May	Aug.	3	None	5	94	5	Leaves Other Total
56..	California-poppy (*Eschscholtzia californica* Cham.).	7 plants	Dec.	Apr.	7	None	5	99	5	All

BORON-TOLERANT

No.	Name	Variety and description	Date planted	Date cropped	Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
57..	Turnip (*Brassica rapa* L.).	Purple-top Globe (6 plants, 10-boron plants damaged).	Dec.	May	6	None	5	115	25	Leaves Roots Total
58..	Common beet (*Beta vulgaris* L.).	Early Wonder (7 plants).	Dec.	Apr.	7	S(?)	5	112	15	Laminae Roots Other Total

See footnotes at end of table.

with other symptoms, under different climatic conditions—Continued

TOLERANT PLANTS—Continued

Dry weight [5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants [6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*°F.*	*Pct.*	*In.*	*Hr.*
53	60	56	50	70	42	Leaves	39	150	283	410	516	701	97.6	64.2	2.14	12.4
75	73	71	63	81	46	Stems	17	22	17	20	28	39				
128	133	127	113	151	88	Weighted mean.	26	80	134	193	254	355				
13	28	28	17	17	13	Leaves	36	158	388	654	854	859	98.4	70.0	1.84	10.3
18	27	31	21	18	13	Stems	11	31	33	55	94	94				
15	25	22	14	13	6	Roots	31	20	47	47	58	52				
46	80	81	52	48	32	Weighted mean.	25	72	160	249	353	397				
60	49	66	59	58	60	Leaves	27	70	116	221	343	582	96.6	68.0	1.94	11.6
24	18	20	19	20	22	Other	19	24	24	49	61	81				
84	67	86	78	78	82	Weighted mean.	25	58	95	179	271	448				
36	45	44	39	33	35	Leaves	43	75	166	353	459	817	96.6	68.0	1.94	11.6
20	19	22	22	17	22	Other	29	28	27	49	58	86				
56	64	66	61	50	57	Weighted mean.	38	61	120	243	323	535				
124	113	127	114	113	93	Laminae	19	72	261	365	474	771	95.6	67.1	1.74	11.5
221	212	222	194	222	152	Other	24	8	35	27	33	68				
345	325	349	308	335	245	Weighted mean.	22	30	117	152	182	335				
133	129	134	133	125	85	All	73	83	151	208	278	456	60.7	69.3	.47	6.0
106	103	111	122	107	65	Leaves	48	150	544	855	1,192	1,416	94.6	64.1	2.03	12.2
19	16	20	24	20	10	Fruit	24	27	60	79	114	131				
141	129	133	138	113	62	Other	16	27	60	57	68	60				
266	248	264	284	240	137	Weighted mean.	29	78	264	402	573	709				
244	239	367	216	187	185	Leaves	34	103	253	531	684	1,168	97.6	64.8	1.95	11.4
284	335	363	260	232	170	Other	38	32	41	46	63	93				
528	574	730	476	419	355	Weighted mean.	36	62	148	266	340	653				
142	128	171	138	114	93	Leaves	54	108	377	705	822	1,073	77.1	71.4	1.24	9.5
						Other	28	39	64	113	153	186				

PLANTS

206	244	359	....	271	284	Leaves	16	65	108	....	213	399	71.9	66.8	0.92	7.7
73	126	110	....	109	66	Roots	19	38	51	....	65	132				
279	370	469	....	380	350	Weighted mean.	17	56	95	....	171	349				
41	28	35	42	34	33	Laminae	25	104	198	346	671	822	78.0	70.3	1.28	9.5
71	67	94	74	70	69	Roots	....	16	16	46	74	72				
31	32	50	45	35	33	Other	20	46	50	94	148	165				
143	127	179	161	139	135	Weighted mean.	12	43	61	138	239	278				

TABLE 1.—*Boron accumulation in various plants and its effect on growth,*

BORON-TOLERANT

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
59	Common beet (*Beta vulgaris* L.).	Early Wonder (7 plants).	May	Aug.	7	S	10	100	10	Laminae Roots Other Total
60	Leaf beet (*Beta vulgaris* var. *cicla* L.).		Oct.	Mar.		W	5	110	25	All
61	Muskmelon (*Cucumis melo* L.).	4 plants cropped soon after early flowering.	June	Aug.	4	D	5	107	5	Leaves Other Total
62	Sweetclover (*Melilotus indica* (L.) All.).	Annual Yellow (15 plants).	Dec.	Apr.	15	None	5	110	10	Leaves Other Total
63	do	do	May	Aug.	15	None	15	124	10	Leaves Other Total
64	Sweet pea (*Lathyrus odoratus* L.).	8 plants	Dec.	Apr.	8	S	10	113	5	Leaves Seed Other Total
65	Sugar beet (*Beta vulgaris* var. *crassa* Alef.).	B. P. I. 2769 (7 plants).	Dec.	Apr.	7	None	5	121	15	Laminae Roots Other Total
66	do	B. P. I. 2769 (5 plants)	Apr.	Sept.	5	S	5	107	10	Tops Roots Total
67	do	B. P. I. 2769 (7 plants).	May	Sept.	7	S	15	117	10	Laminae Roots Other Total
68	do	U.S. No. 1 (7 plants).	June	Aug.	7	S	10	146	10	Laminae Roots Other Total
69	Oxalis (*Oxalis bowiei* Herb.).	Bowie (5 plants from tubers).	Oct.	Mar.	5	S	10	121	None	All

See footnotes at end of table.

with other symptoms, under different climatic conditions—Continued

PLANTS—Continued

Dry weight [5] of plants grown in solution with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants [6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	°*F.*	*Pct.*	*In.*	*Hr.*
30	52	43	62	46	54	Laminae	52	150	370	637	986	1,263	99.4	59.4	2.21	12.0
72	124	132	137	113	121	Roots	33	46	46	44	63	82				
22	42	39	49	36	45	Other	35	60	76	74	131	152				
124	218	214	248	195	220	Weighted mean.	38	74	117	198	293	386				
189	254	312	276	248	170	All	16	58	66	82	139	200	64.0	69.8	.73	6.9
63	87	94	89	87	77	Leaves	37	223	923	1,621	2,181	3,875	98.6	64.2	1.96	11.5
47	66	84	72	63	69	Other	16	46	90	163	253	373				
110	153	178	161	150	146	Weighted mean.	28	147	530	969	1,371	2,220				
34	60	79	65	54	64	Leaves	64	214	363	602	1,089	1,438	78.0	70.3	1.28	9.5
64	100	132	97	102	103	Other	6	8	33	38	176	297				
98	160	211	162	156	167	Weighted mean.	26	85	157	264	492	734				
18	21	22	21	30	23	Leaves	58	349	665	1,164	1,597	2,245	99.4	59.4	2.21	12.0
28	31	39	34	47	37	Other	25	36	39	33	47	83				
46	52	61	55	77	60	Weighted mean.	38	162	265	465	651	912				
24	32	35	34	27	26	Leaves	30	190	520	979	1,084	1,551	78.0	70.3	1.28	9.5
5	8	5	4	3	1	Seed	1	18	50	55	..					
48	55	73	76	65	74	Other	36	33	74	156	220	352				
77	95	113	114	95	101	Weighted mean.	32	85	211	398	474	664				
43	36	41	43	42	45	Laminae	20	102	143	426	521	668	77.1	71.9	1.24	9.4
44	42	66	65	50	50	Roots	2	6	6	20	31	28				
64	63	81	81	78	67	Other	8	28	58	82	105	166				
151	141	188	189	170	162	Weighted mean.	10	40	58	139	186	263				
39	217	202	183	218	173	Tops	20	89	177	262	304	592	96.5	67.5	2.04	10.2
35	174	242	192	217	207	Roots	6	28	28	38	33	55				
74	391	444	375	435	380	Weighted mean.	13	62	96	147	169	299				
67	106	137	110	152	104	Laminae	35	122	286	612	686	1,008	97.3	70.0	1.91	11.0
51	180	223	151	265	215	Roots	20	13	24	52	22	41				
63	132	153	126	144	148	Other	42	35	46	82	68	133				
181	418	513	387	561	467	Weighted mean.	33	48	101	221	214	286				
41	51	60	77	77	88	Laminae	19	106	234	495	588	975	97.8	63.8	1.76	11.5
40	54	67	93	71	85	Roots	19	27	24	30	38	46				
43	69	86	121	109	107	Other	16	49	49	72	65	88				
124	174	213	291	257	280	Weighted mean.	18	59	93	171	214	354				
99	128	152	165	149	104	All	16	65	264	426	655	1,191	74.8	62.5	.78	6.6

TABLE 1.—*Boron accumulation in various plants and its effect on growth,* BORON-TOLERANT

Crop					Plants cropped	Boron deficiency symptoms with trace boron[1]	Boron concentration for best growth[2]	Relative tolerance[3]	Lowest concentration for injury[4]	Part weighed
No.	Name	Variety and description	Date planted	Date cropped						
					No.		*P. p. m.*	*Pct.*	*P. p. m.*	
70	Cotton (*Gossypium hirsutum* L.).	Upland, Acala (4 plants).	May	Nov.	4	S	10	130	10	Leaves Green bolls Seed cotton Other
										Total
71	Artichoke (*Cynara scolymus* L.).	2 plants	Oct.	Mar.	2	S	5	123	5	Laminae Other
										Total
72	Asparagus (*Asparagus officinalis* L.).	5 seedling transplants; tops cut in October; plants cropped in May.	Apr.	Oct. May	5	S	15	217	25	Tops do Roots
										Total

[1] S=presence of leaf or other morphological abnormalities; M=time of flowering or ripening substantially affected; D=trace-boron plants more severely attacked by mildew than plus-boron plants; W=severe wilting.

[2] Indicates boron concentration producing greatest total dry weight of plants; the differences between the successively higher concentrations were often small or inconsistent.

[3] Computed by multiplying average weight of plants in 5-, 10-, and 15-boron by 100 and dividing by weight of plants in trace- or 1-boron, whichever of these two was the greater.

SYMPTOMS AND OTHER PLANT REACTIONS

BLACKBERRY (1)[5]—The mesophyll of the leaves in trace-boron was mildly buckled, the terminal buds of the canes had lost their dominance, and by the end of the season numerous short branches had developed near the tips. The older leaves on the 1-boron canes showed slight marginal burning, but otherwise the plants were normal in appearance. Little growth occurred in 5-boron, and the plants in the higher concentrations died early in the season.

LEMON (2).—Deficiency symptoms were absent from trace-boron. The older 1-boron leaves yellowed and burned along the margins; the yellowing extended inward between the veins (pl. 1, *A*). The plants in 5-boron were in very poor condition, and those in 10-, 15-, and 25-boron did not survive.

ELM (3).—Many of the trace-boron elm leaves showed late-season yellowing with occasional dead areas along the margins. Only a few of the 1-boron leaves showed marginal burning, but all 5-boron leaves were severely burned, the necrotic areas extending inward between the veins toward the midrib (pl. 2, *A*).

CHERRY (4).—Leaves that developed on the trace-boron plants during the midsummer months were chlorotic, many of them being green only along the veins, and the marginal serrations were resinous to dead. There were a number of dead leaf tips in 5-boron, but cherry.

---

[5] Numbers in parentheses refer to table 1.

with other symptoms, under different climatic conditions—Continued

PLANTS—Continued

Dry weight[5] of plants grown in solutions with indicated concentration of boron (p. p. m.)						Part analyzed	Boron in plants[6] grown in solution with indicated concentration of boron (p. p. m.)						Average climatic conditions during last half of growth period			
Trace	1	5	10	15	25		Trace	1	5	10	15	25	Maximum temperature	Relative humidity	Evaporation per week	Sunshine
Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*°F.*	*Pct.*	*In.*	*Hr.*
81	113	131	179	111	124	Leaves	16	187	306	522	833	1,625	88.4	69.3	1.34	9.1
16	111	138	199	108	118	Stems & roots	15	24	32	33	48	60				
0	38	20	27	16	32											
107	200	303	367	204	249											
204	462	592	772	439	523											
333	454	510	512	510	423	Laminae	38	112	236	440	830	1,358	73.9	62.2	.73	6.5
282	385	571	482	499	361	Other	16	58	82	66	140	182				
615	839	1,081	994	1,009	784	Weighted mean.	28	87	155	259	489	817				
42	102	183		269	210	Tops	55	74	140		244	288	91.6	69.2	1.39	9.4
14	55	78		163	90	do	43	54	59		170	175	72.7	65.3	1.02	8.2
22	73	79		226	83	Roots	13	16	30		27	59				
78	230	340		658	383											

---

[4] First evidence at time of cropping of yellowed or burned leaves, marginal restrictions, or other abnormalities.

[5] Oven-dry weight. [6] Expressed on oven-dry basis.

[7] The boron concentration in each plant part was multiplied by the weight of that part, and the total of these products was divided by the sum of the weights of the plant parts.

[8] Value omitted because of abnormal growth of trace-boron plants.

as observed in other tests, does not show much leaf injury when grown in concentrations of boron sufficiently high to cause growth depression.

Peach (5).—Deficiency symptoms were lacking in trace-boron of this planting, but in the 1929 experiment the leaves tended to be chlorotic. The 1-boron leaves were normal and the 5-boron leaves appeared so, but the older ones abscised earlier than those in trace-boron and 1-boron. In keeping with observations made in other experiments and in the field, necrotic lesions developed in the 5-boron stem bark. These lesions commonly appear above the leaf axils.

Persimmon (6).—The margins of the trace-boron leaves were necrotic to dead. Associated with the relatively high boron accumulation, the leaf margins in 1-boron were cupped downward and yellowed or burned. The 5-boron leaves were severely cupped and burned, yellow areas appearing inward from the margins between the veins (pl. 1, *B*).

Fig (7).—The terminal buds of the trace-boron plants became dormant before the end of the season; there were numerous small branches near the tops of the plants; the successive main-stalk internodes were shorter as the season progressed; the late-season leaves were misshapen, the mesophyll was buckled, and there were large irregular chlorotic and partly dead areas extending inward along the veins from the margins of the leaves. The terminal buds of the 1-boron trees were still growing late in the season, and the leaves and branching habits were normal. All but the upper third of the leaves in 5-boron were burned along the margins. The average height of plants in trace-, 1-, 5-, and 10-boron was 85, 210, 125, and 11 cm. respectively.

STRAWBERRY (8).—Deficiency symptoms were lacking in the trace-boron plants, and these plants were equal in appearance to those growing in 1-boron. The trace- and 1-boron beds each produced about 20 fruits. When cropped late in March, only a single leaf was alive on each of the 5-boron plants.

LUPINE (9).—There were no deficiency symptoms in the trace-boron plants, but flowering was 3 weeks later than in the 1-boron culture. The leaves on both the 5- and the 10-boron plants were severely burned. All plants in 15- and 25-boron died.

GRAPE (10, 11).—The Malaga grapes grown in 1930 (10) and the Sultanina grapes grown in 1931 (11) reacted similarly. The trace-boron leaves were buckled and had irregular dead areas along the margins. The 1-boron leaves were entirely normal, whereas the 5-boron leaves were reduced in size, the margins were restricted, and there were numerous resinous necrotic patches and spots along the margins. Little of the older leaf tissue of the 10-boron plants was alive at the end of the season. The total length of the Malaga shoots in trace-, 1-, 5-, 10-, and 15-boron was 528, 1,505, 310, 155, and 63 cm. respectively.

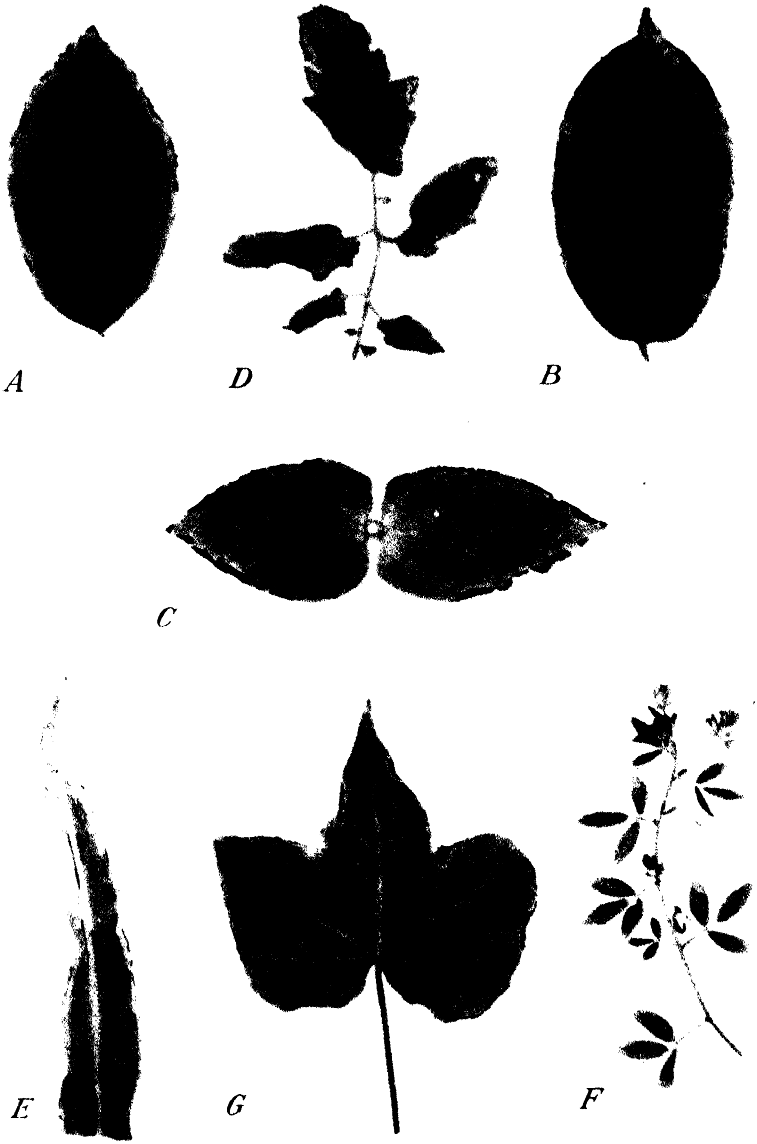
VIOLET (12).—There were no deficiency symptoms in trace-boron, the 1-boron plants were likewise normal, the leaves of the 5-boron plants were curled downward at the margins, those of the10-boron were badly burned, and only a few small leaves remained alive in 15-boron until March.

PANSY (13).—The trace-boron pansies were without symptoms of deficiency and, like the violet, this plant proved to be highly sensitive to boron. The edges of the older 5-boron leaves were restricted and had white or burned margins. Marked boron injury was evident even in the youngest leaves of the 10-boron plants, the 15-boron plants were in poor condition and produced few flowers, and all of the 25-boron plants died.

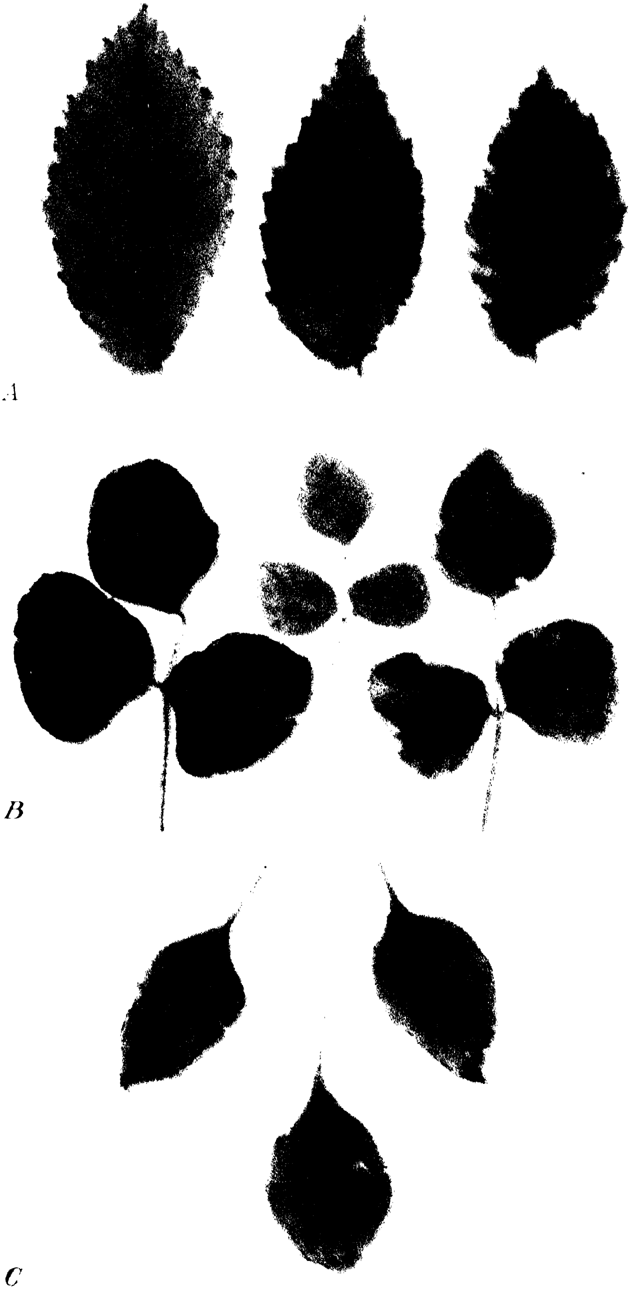
KIDNEY BEAN (14, 15).—Symptoms of diagnostic significance were lacking in trace-boron of both of these plantings. In 1930 (14) only a few green pods were formed by the trace-boron plants, whereas in 1933 (15) the trace-boron plants were relatively fruitful. In 1933 (15) 50 percent of the trace-boron leaves had late-season mildew, only about 5 percent had mildew in 1- and 5-boron, and none in 10-boron. Mild marginal yellowing, burning, and cupping were noted in the 1-boron plants in both years; the injury was severe in 5-boron, and nearly all leaves fell from the 10-boron plants.

COWPEA (16).—Deficiency symptoms were lacking in trace-boron, but the lower leaves developed much mildew just prior to cropping. A few old leaves had mildew in 1-boron, but mildew was absent from 5-, 10-, and 15-boron. The 1-boron plants did not show leaf injury, but the older leaves dropped earlier than in trace-boron. In 5-boron 50 percent of the leaves showed marginal burning; in 10-boron the margins of all leaves were constricted, and all but the youngest showed marginal burning; in 15-boron the plants were severely injured; and the 25-boron plants died (pl. 2, *B*).

JERUSALEM-ARTICHOKE (17).—Deficiency symptoms were lacking in trace-boron. The lower 60 percent of the 1-boron leaves showed



Leaves of various plants, showing symptoms of boron injury. *A*, Lemon. *B*, Persimmon. *C*, Zinnia. *D*, Tomato; this example is highly typical of more advanced injury, which in all respects is like that shown by potato. *E*, Milo; the injury shown by corn is similar. *F*, Alfalfa; not infrequently the chlorotic marginal areas are more sharply defined and have less yellow. *G*, Cotton; in more severe cases yellowing and burned areas are found extending inward from the margins between the larger veins.


A
B
C

some marginal burning with necrotic spots between the veins, but nearly all of these leaves were partly alive when cropped. In 5-boron the leaves on the lower 75 percent of the stalk died, injury was still more severe in 10-boron, and only a few leaves remained alive on the 15-boron plants. Seven seedlings of the deodar cedar (*Cedrus deodara* Laws.) were set in 25-boron in place of the artichoke and made a creditable growth.

LARKSPUR (18).—The trace-boron stalks did not elongate properly, their terminals aborted or died, and the apical portions of many of the leaves became chlorotic or died. The 1-boron plants were normal. Some burning occurred on leaves halfway up the stalks of the 5-boron plants, and bud formation was delayed. These symptoms were progressively more marked in 10-boron, and the flower buds developed poorly. There were few flowers and much leaf abscission on the 15-boron plants, and the 25-boron plants were in poor condition.

ZINNIA (19).—The trace-boron plants were normal. Mild burning occurred along the margins of the 1-boron leaves, and the burning became progressively more severe through each of the successively higher concentrations (pl. 1, *C*).

BARLEY (20).—Boron-deficiency symptoms of morphological character were not in evidence in the trace-boron barley. There were mild symptoms of injury in 5-boron and these became progressively more severe through to 25-boron. In both summer and winter plantings in 1929 the growth of barley in trace-boron was substantially below that in 5-boron, but in both of these plantings mildew was severe on the trace-boron plants. Boron injury in barley is shown by burning of the margins and tips of the leaves. Farther down from the tips, in advance of the mass burning of the tissue, there is some irregular loss of chlorophyll, and small necrotic patches appear between the veins. Barley grown in trace-boron in 1929 was severely attacked by mildew (*Erysiphe graminis* DC.), the amount becoming less as the concentration of boron was increased (*4*); a differential susceptibility to mildew was observed in this planting of 1932, but the attack was not severe even in trace-boron. The spot blotch disease (*Helminthosporium sativum* Pam., King, and Bak.) did not occur in the trace-boron culture but was present in 1-boron and became more severe as the boron concentrations increased. The fact that Christensen (*3*) failed to confirm the writer's observations on the relation between boron concentration and the incidence of disease might represent either differences in barley varieties or in the strains of the organisms; he indicated difficulty in differentiating between boron injury and lesions produced by other causes, but this is hard to understand since boron injury to barley could hardly be confused with spot blotch for the reason that the latter attacks the entire leaf, whereas boron injury is most severe at the leaf tips. Wheat grown in the preliminary experiments in 1929 showed boron reactions similar to those of barley, but mildew was equally severe under all the treatments.

EXPLANATORY LEGEND OF PLATE 2

Leaves of three plants, showing symptoms of boron injury. *A*, Elm; because of its sensitiveness to boron injury and its characteristic markings, the elm is a good boron-indicator plant. *B*, Cowpea; yellowing of young leaves is not always a characteristic of boron injury in this plant or in the similarly sensitive bean; the latter does not always show the pronounced resinous spotting. *C*, Redpepper.

PEA (21, 22).—Symptoms of boron deficiency were lacking in the trace-boron cultures. There was a little leaf injury in 1-boron, and the symptoms became more severe through the remaining cultures.

LIMA BEAN (23).—The tips of the shoots died in trace-boron and some of the leaves showed scattered dead areas. A number of the leaves in 1-boron showed a trace of boron injury, and the marginal burning in 5-boron was marked. The 10-boron plants were badly injured. The 15-boron plants produced no pods, and approximately half of the leaf area was dead when the plants were cropped. There was almost no growth in 25-boron. Three of the five plants in 1-boron were injured mechanically, and the weight (76 gm.) is omitted from table 1.

SWEETPOTATO (24).—The foliage of the trace-boron plants showed no deficiency symptoms, but the tubers were greatly elongated and slender, and none of them would have been marketable. The experiment indicates that tuber shape in the sweetpotato is a function of boron concentration. Tuber diameter, relative to tuber length, increased through the series of treatments, some of the tubers in 25-boron being nearly spherical. The number of marketable tubers in the six cultures was respectively 0, 16, 15, 23, 8, and 3. The greatest weight of roots was in trace-boron. The oldest leaves in 5-boron were burned along the margins, and the injury increased through 10- and 15-boron; in 25-boron all but the youngest leaves showed severe injury.

ONION (25–27).—No deficiency symptoms were observed in the trace-boron plants of either the winter (25) or the summer (26 and 27) plantings. The tips of leaves in 1-boron were burned lightly; the amount of burning increased until, in 25-boron, the leaves were burned back from the tips for 5 to 15 cm. The greatest growth of plants occurred in trace-boron in each of the plantings. It is to be noted that the winter plants accumulated much more boron in their roots, both actually and relatively compared to that in the tops, than did the summer plants.

CARROT (28, 29).—In trace-boron, no deficiency symptoms developed in the plants of either the winter (28) or the summer (29) planting. Injury appeared in a few leaves in the 10-boron plants of the winter planting. In the summer planting a similar amount of injury appeared in the 5-boron plants. The youngest leaves showed mild injury in the 25-boron plants of the winter planting, and marked injury occurred in the corresponding plants of the summer planting.

REDPEPPER (30).—Deficiency symptoms were lacking in trace-boron. The margins of the older leaves in 5-boron were restricted and turned downward. Injury was successively more severe in 10- and 15-boron; and in 25-boron there was a marked reduction in leaf size, accompanied by cupping of entire leaves, with severe marginal burning (pl. 2, *C*).

KENTUCKY BLUEGRASS (31).—Deficiency symptoms were lacking in trace-boron, and the tips of the leaves were green. In 1-boron there was a little mild tip burning that became successively more marked through the series, but this burning was not severe even in 25-boron. The entire plants of the June 1 cropping contained several times as high concentrations of boron as the leaves of the May 4 cropping, indicating a more rapid accumulation of boron during the latter period.

Corn (32, 33).—Morphological symptoms of boron deficiency were not evident in trace-boron. The trace-boron plants (33) matured more slowly than did the plants in the plus-boron cultures. (See milo under 36 and 37 in this connection.) The boron injury of corn resembles that of milo (pl. 1, *E*) and is shown by yellowing between the veins, particularly toward the tips of the leaves, followed and accompanied by marginal and apical burning. Boron injury, first evident in 5-boron, increased to severe in 25-boron.

Potato (34).—The mesophyll of the trace-boron potato leaves buckled, and the oldest ones were still green when cropped. The oldest leaves in 1-boron showed slight marginal burning, the injury increasing to severe in 25-boron, where more than 50 percent of the leaf tissue was burned and all leaves were cupped. Boron injury in the potato is much like that in the tomato (pl. 1, *D*). The tubers in 25-boron showed some russeting, and these tubers were the only ones entirely free from rhizoctonia. Idaho Russet potatoes planted December 10, 1929, and cropped May 24, 1930, had dry weights in trace-, 5-, 10-, 15-, and 25-boron, respectively, as follows: Tops 21, 31, 37, 35, and 35 gm.; and tubers 88, 117, 172, 82, and 53 gm.

Cabbage (35).—Deficiency symptoms accompanying the reduction in growth were not in evidence in trace-boron. Some marginal restriction of the leaves in 10-boron was evident. This was more marked in 15-boron, and in 25-boron there was a little mild burning. The marked difference in boron accumulation shown in the covered and exposed leaves is doubtless due to differences both in age and in exposure.

Milo (36, 37).—Morphological symptoms of boron deficiency were lacking in trace-boron, but the trace-boron milo (36) continued to grow and send out new shoots from many nodes after the plus-boron plants had matured. In the second planting (37) the same maturity effect was observed in trade-boron, but because of late planting there was little gain in plant weight. A tendency toward extensive tillering has been noted in boron-deficient cereal cultures by Sommer (*11*), Morris (*10*), and Warington (*13*). The fact that the 25-boron milo plants also exhibited a tendency toward delayed maturity suggests that either a limited supply or a large excess of boron causes milo to behave like sorgo and sugarcane as regards longevity and the translocation of carbohydrates for seed production. Some burning of the leaves developed in 5-boron, the injury increasing to severe in 25-boron (pl. 1, *E*).

Calendula (38).—The trace-boron and 1-boron plants were without symptoms. The 5-boron leaves were cupped downward at the margins with a little marginal yellowing. More severe injury resulted in 10- and 15-boron, with severe burning of old leaves and cupping of young ones in 25-boron.

Radish (39–42).—The trace-boron radishes cropped in February (39) were reduced in size, but splitting was limited to the tips of the fleshy roots; those cropped in November (40) were more angular than those in the plus-boron beds but otherwise were normal; the March (41) radishes were irregularly shaped, the larger ones were split and on standing overnight became spongy, whereas the plus-boron radishes did not; the July (42) trace-boron radishes were reduced in size but otherwise appeared normal. In a winter planting of 1929, the trace-boron radishes weighed only half as much as the 5-boron and there was much splitting. Mild symptoms of boron injury in the form of marginal burning were shown by the leaves in 10-boron in all plantings, and this injury increased to marked or severe in 25-boron. The extent of boron accumulation both in roots and tops in the higher boron beds corresponds in order of rank with hours of sunshine per day better than with maximum temperature, relative humidity, or evaporation.

Oats (43).—Deficiency symptoms were lacking in trace-boron. The planting was cropped when it had just started to ripen, because of rank growth and lodging. The boron injury was mild in 5-boron, increasing to severe in 25-boron.

Celery (44).—The hearts of all the trace-boron plants were badly rotted, and the petioles were discolored and transversely cracked on the lower surface; only the older leaves were alive when the plants were cropped. The leaves of the 25-boron plants were reduced in size and thickened, but there was no burning. The 1-, 5-, 10-, and 15-boron plants were essentially equal in appearance, and all had flower stalks upward to 60 cm. tall.

Mustard (45).—Deficiency symptoms were not observed in trace-boron. Mild boron injury, represented by marginal yellowing of the leaves, was shown in 5-boron; the injury increased to 25-boron, where 60 percent of the leaves had restricted, yellowed, and burned margins.

Parsley (46).—The trace-, 1-, 5-, and 10-boron plants were essentially alike and showed no deficit or injury symptoms; all were vigorous, bushy, compact plants about 40 cm. tall. The 15-boron plants were uniformly reduced in size (30 cm. tall) but showed no leaf injury. The 25-boron plants were without leaf markings and all remained alive, but they were very small.

Alfalfa (47–49).—The characteristic yellowing of boron-deficient alfalfa was not noted in these plantings, but in a summer planting in 1929 the terminal growth was notably yellowed and the trace-boron growth was poor. In the July cutting of 1931 (48), there was little or no growth depression in trace-boron and the stand was maintained, but by the final cropping in September only 16 of the original 20 plants were alive. Loss of stand also occurred in higher boron concentrations both in this planting and in the preceding winter planting (47). In 15- and 25-boron the margins and tips of older leaves yellowed and occasionally burned (pl. 1, *F*).

Lettuce (50, 51).—There were no deficiency symptoms in either of the varieties of lettuce in these summer plantings, and flowering was not delayed. In an earlier winter planting that rotted out during a period of rainy weather, the growth in trace-boron was very poor and only fair in 1-boron, whereas good heads were forming in 5-boron. These summer plantings (50, 51) developed mild marginal burning in 1-boron, the injury increasing to 25-boron. In the latter concentration the margins of all leaves were burned, the older ones severely so.

Tobacco (52).—No deficiency symptoms were apparent in the trace-boron plants. Marginal burning with interveinal yellowing occurred in 10-boron, and in 25-boron the leaves were cupped, with marginal burning and dead spots between the veins. Intermediate injury developed in 15-boron.

Vetch (53).—Deficiency symptoms were lacking in trace-boron. The oldest leaves showed a little boron injury in 5-boron; injury increased through 10- and 15-boron; and in 25-boron even the youngest leaves burned. No nodules developed on the roots in trace-boron; they were few in 1-boron, abundant in 5-boron, reasonably so in 10-boron, few in 15-boron, and none in 25-boron.

TOMATO (54, 55).—There was no evidence of deficiency symptoms in the trace-boron plants of either of these plantings. Mild boron injury was evident in 5-boron, and the injury increased to 25-boron, where the leaves were reduced in size, cupped, and burned (pl. 1, *D*).

CALIFORNIA-POPPY (56).—Boron-deficiency symptoms were lacking in trace-boron. The 1-boron plants were likewise normal. A few 5-boron leaves showed mild injury, and the injury increased to 25-boron, where it was substantial. The burned tissue turned black, a reaction not observed among the other plants tested.

TURNIP (57).—Boron-deficiency symptoms were lacking or uncertain in trace-boron. There was only mild leaf injury in 25-boron. The plants in 10-boron made a poor start and were irregular when cropped.

COMMON BEET (58, 59).—Deficiency symptoms in the winter-grown beets (58) were limited to a shallow crack in one root. The summer beets showed marked deficiency; the leaves were small, one root had heart rot, and two had advanced dry rot. Only obscure injury was observed in the leaves of the winter 15-boron plants (58), and the injury was mild in 25-boron. Leaf injury was evident in the 10-boron plants of the summer planting (59), increasing to pronounced in 25-boron.

LEAF BEET, OR CHARD (60).—Though without morphological symptoms of boron deficiency, the trace-boron chard wilted repeatedly during the middle of the warmer days, but no wilting was observed in the plus-boron plants. Beets in trace-boron occasionally reacted similarly, but the wilting was never so pronounced as in the chard. In contrast to this observation in chard and beets, Warington (*13*) has reported that the broadbean (*Vicia faba* L.) remained turgid in minus-boron cultures at times when plus-boron plants wilted. The older chard leaves in 25-boron were restricted at the margins and showed mild burning.

MUSKMELON (61).—Mildew was severe in trace-boron, decreasing to very little in 10-boron, with none in 15- or 25-boron. There were many dead and partly dead leaves in trace-boron but fewer in 1-boron; this difference may have been due to the difference in mildew. A few 5-boron leaves showed a little boron injury, and the injury increased to 25-boron, where all leaves were cupped and the older ones badly burned.

SWEETCLOVER (62, 63).—Deficiency symptoms were absent from trace-boron. Injury was first apparent in the 10-boron plants of both the winter and the summer plantings, but in neither planting did it become severe in 15- and 25-boron.

SWEET PEA (64).—Occasional dead areas developed in the trace-boron leaves, but the plants were otherwise normal. Mild injury symptoms occurred in 5-boron, and the injury though marked was not severe even in 25-boron.

SUGAR BEET (65–68).—Boron-deficiency symptoms of varied severity developed in the trace-boron cultures in the summer plantings of 1930, 1931, and 1933 (66–68), but the beets grown during the winter of 1930–31 (65) were normal. Boron deficiency in beets, as is now well known, results in a dry rot of the roots and a heart rot at the crown of the plant, and in addition it sometimes kills the young leaves or prevents their growth. It may result, as in crop 66 (fig. 2), in the death of the older leaves, where deterioration typically starts at the margins and progresses inward. The mesophyll of the leaves of affected plants is notably buckled. The foregoing disorders of the beet were first ascribed to boron deficiency by Brandenburg (*1*) in 1931. Injury due to excessive boron was shown in 25-boron by restriction and burning of the leaf margins, but in no instance was the injury severe.

Oxalis (69).—Many leaves in trace-boron were semichlorotic and exhibited marginal abnormalities, necrotic spots appearing in the

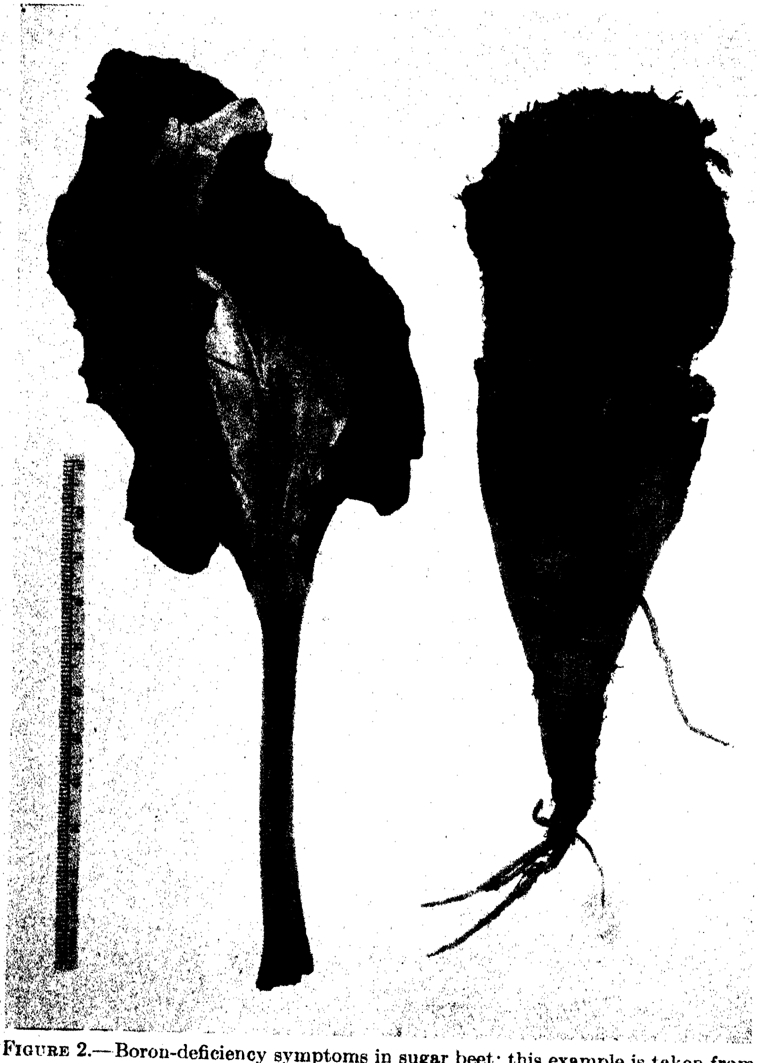


Figure 2.—Boron-deficiency symptoms in sugar beet; this example is taken from the plants reported under crop 66.

mesophyll. There were no morphological symptoms of boron injury in any culture.

COTTON (70).—The trace-boron cotton leaves both in this planting and in a 1929 planting were characterized by chlorotic blotches, large irregular dead areas, and buckled mesophyll. The plants had a compact appearance due to reduced branch lengths, and few or no bolls were set until October. Mild marginal burning of the leaves occurred in 10-boron, and the injury to leaves increased to severe in 25-boron (pl. 1, *G*). The numerical formula here employed for measuring relative tolerance is not very well suited to plants such as cotton that make substantially more growth with 5 or 10 p. p. m. of boron than in trace or 1 p. p. m. but which are substantially injured in 15 or 25 p. p. m. As previously noted, the reactions of a 1929 planting of cotton paralleled this test, but in a 1931 planting that had to be cropped in September before any bolls had matured, the trace-boron plants started to set bolls in late August. The boron content of these trace-boron plants was not out of line with that of the other two plantings.

ARTICHOKE (71).—The trace-boron plants were chlorotic, the petioles broke easily and clean, the floral stalks did not elongate normally, and the floral buds were about 2 cm. in diameter. The color of the 1-boron plants was excellent, the main floral buds were 6 cm. in diameter, and the flower stalks were 30 and 50 cm. tall; each plant had 3 secondary buds. The 5-boron plants were larger than the 1-boron, the main buds were 8 cm. in diameter, and the flower stalks were each 60 cm. tall. Mild boron injury appeared in the older leaves. The 10-boron plants produced buds 3 and 5 cm. in diameter on 40-cm. stalks; boron injury was marked but confined to the older leaves. The bud development in 15-boron was similar to that in 10-boron, and some injury was evident on half of the leaves. The 25-boron plants and their floral buds were further reduced in size, and as much as 60 percent of the leaf tissue of the older leaves was burned. The data indicate that this plant might respond to boron under field conditions. The fact that artichokes do remarkably well in the Half Moon Bay area of the central California coast supports the evidence drawn from other crops that boron requirements are lower under conditions of reduced light intensity. In this coastal area the skies are overcast much of the time.

ASPARAGUS (72).—The plants growing in trace-boron were small; there were many dead stems, and one plant died. There was some boron injury in the tips of plants growing in 25-boron. Only one of the five plants in 10-boron made a satisfactory start, and those results are accordingly omitted from table 1. The approximate average height of plants in the six cultures was respectively 40, 60, 80, 65, 100, and 80 cm. Asparagus plants remained alive for 3 years in a culture supplied with solution containing 100 p. p. m. of boron.

DISCUSSION

The data on the reactions of the 50 botanical species and varieties of plants to boron, presented in table 1 and in the notes on plant symptoms, provide a substantial basis for the conclusion that there is some overlapping of the beneficial and injurious effects of boron within plants. At least mild leaf injury was observed in 19 of the

72 plantings at or below the boron concentrations that resulted in greatest growth. This finding is entirely compatible with the view that boron is carried into the leaf in the transpiration stream and that from the leaves there is little re-movement of boron with the sugars and other compounds passing out of the leaves to the fruit and other tissues (stone fruits are known to be an exception). So much boron may accumulate in the older leaves of a plant that yellowing or burning results, while at the same time the boron supply to actively enlarging meristematic tissues is insufficient for their most rapid growth.

Because of the indications of an overlapping between the beneficial and injurious effects of boron within a plant, any conception of an optimal concentration in the substrate becomes theoretical and is certainly relative to other conditions. It is a matter of convenience, notwithstanding, to review the data presented in table 1 under the conventional headings Boron Deficiency and Boron Toxicity.

Of the 72 croppings, including the repetition of varieties grown in more than one season, the best growth occurred in the respective beds the following number of times:

Boron concentration (p. p. m.):	*Distribution of best growth*
Trace	19
1	20
5	20
10	7
15	6
25	—

Thus over 70 percent of the plantings responded to more than a trace (0.03 to 0.04 p. p. m.) of boron, and about 45 percent of the varieties made better growth with 5 p. p. m. or more of boron than with less than that amount.

BORON DEFICIENCY

Of the 58 varieties of plants, 20 developed morphological symptoms of deficiency; the maturity of 4 was prominently affected; and 4 were more subject to mildew (*3*, *4*, *16*) in the trace-boron culture than in the higher concentrations.

Boron deficiency in barley was shown by a notable susceptibility to mildew; in corn, by delayed maturity; in milo, by continued vegetative growth after maturity had occurred in the intermediate boron treatments; in alfalfa, by loss of stand and reduced growth with or without yellowing of upper leaves; in artichokes, by chlorotic, undersized plants, brittle petioles, and repressed floral development; in asparagus, by reduced growth and death of stems; in lima beans, by death of plant tips and dead areas in leaves; in kidney beans, in one crop, by poor development of pods and root nodules and susceptibility to mildew; in cowpeas, by mildew; in beets, by deterioration of old leaves, dry rot, heart rot, and death of some plants; in celery, by rotting of hearts and by transverse cracking and discoloration of petioles; in leaf beets, by daily wilting; in cotton, by chlorotic blotches, buckling, and dead areas in the mesophyll, and by extensive abscission of floral buds or by delayed fruiting; in muskmelons, by excessive mildew; in potatoes, by buckling of mesophyll; in radishes, by irregularly shaped and spongy storage roots with splitting of the larger ones; in sweetpotatoes, by slender and greatly elongated tubers; in sweet peas, by necrotic spotting of leaves; in blackberry, by buckled mesophyll and loss of dominance of the terminal buds; in cherry, by chlorotic midsummer leaves with tips of serrations resinous or dead; in the elm, by yellowing of leaves late in the season with dead areas along the margins; in the fig, by shortened internodes, chlorotic and misshapen leaves with dead areas, and loss of dominance of the terminal bud; in grapes, by buckled mesophyll and irregular dead areas along the leaf margins; in the persimmon, by dead or chlorotic leaf margins; and in the date palm (not included in table 1), by lack of stiffness and finer growth of fronds.

The extent to which symptoms were developed and growth was curtailed by boron deficiency was often at variance when the same variety was replanted, not only as between winter and summer plantings, but also as between successive summer or winter plantings. Sugar beets (table 1, crops 65 to 68), as grown during each of three summers, were markedly deficient in the trace-boron culture, but in a winter planting there was no evidence of deficiency. Similarly, common beets (crops 58 and 59) were deficient in a summer trace-boron planting and normal in a winter planting. Radishes (crops 39 to 42), on the other hand, were normal other than for size in a summer planting, angular in a fall planting, and showed deficiency symptoms of the fleshy roots in each of two winter plantings. Alfalfa (crops 47 to 49) developed no marked deficiency in either the winter or summer planting in 1931 but was markedly deficient in the summer of 1929. Onions were normal in the trace-boron culture irrespective of time of planting or harvest.

The lack of uniformity in the response of a number of the plants to trace-boron resulted in a reluctance on the part of the writer to publish the data without some explanation. In attempting to account for the apparent discrepancies, consideration was first given to the possibility that some other element, introduced with the nutrient salts as an impurity, replaced boron. Greenhouse investigations, in which cotton was the principal test plant, involved trials with a series of elements, including many of those tested by Brenchley and Warington (*2*) and, in addition, gallium, scandium, germanium, and indium. With the possible exception of scandium, the results were negative. Scandium sometimes seemed to give a slight response, and it has since been learned that the salt employed was high in magnesium and contained only a little scandium. Spectroscopic examination of the culture-solution salts and plant material likewise failed to provide evidence that other elements were involved as a cause for the differences.

An explanation for the seasonal differences in boron requirements was also sought in another direction. As reported elsewhere (*6*) the growth of cotton seedlings in minus-boron solutions was found to be improved and the severity of certain of the boron-deficiency symptoms reduced when small amounts of indole-3-acetic acid were added daily to the nutrient solution. The advantageous effects of the indole-3-acetic acid were most marked when the plants were grown under reduced light. Although these results might be interpreted as indicating that boron is essential to the formation of auxin in plants, an alternate suggestion is that the indole-3-acetic acid increased the movement of the traces of boron from the cotyledons to the meristematic tissues.

Evidence that photoperiod reactions are not primarily involved is supplied by the work of Warington (*13, p. 455*), who reached the following conclusion:

> Within the range of 7–16 hours, the length of day has no bearing on the need of the plant for boron, since with one possible exception, where the case remained unproven * * * the deficiency symptoms characteristic of a lack of boron were similar under both long and short day conditions, although they were less pronounced and their rate of progress retarded if the days were short.

In Warington's work the day length was shortened by placing plants in dark chambers overnight with temperatures and midday light intensities unchanged. That work accordingly does not provide an opportunity for specific deductions on relations between high light intensities and the boron requirements of plants; the differences found by Warington in the time of appearance of symptoms are, nevertheless, in the direction to be expected on the basis of the light-auxin relationships cited above. This subject will receive further consideration in the section dealing with the movement of boron in plants.

A direct analysis of the data of table 1 for relations between boron deficiencies and average light intensities is not possible, as the average number of hours of sunshine per day does not constitute a satisfactory index to light intensity, particularly when different summers or different winters are compared in a region where thinly overcast skies (high fogs) occur frequently but irregularly during the morning hours.

BORON TOXICITY

In table 1 the plantings are arranged in an order related to their relative tolerance to boron. To determine this order, tolerance was estimated by dividing the average of the plant weights in 5-, 10-, and 15-boron by the weight in trace- or 1-boron, whichever of the two was the higher; the quotient (×100) is recorded as relative tolerance. The first nine plants of the series did not survive in boron concentrations as high as 15 p. p. m., and for this reason their tolerances are not indicated numerically. These plants are given a position in the table on the basis of relative growth in trace-, 1-, and 5-boron. The foregoing procedure is quite arbitrary, and neither the positions of the plants in the table nor the numerical values can be regarded as highly definite. The prevailing climatic conditions during growth had marked effects on the reactions of the plants to boron, and the same plant reactions did not result when a plant was grown during each of several seasons. The position of a plant such as cotton, for example, which grew best in a number of seasons in 10-boron, is too far along in the series if its behavior in 15- and 25-boron is compared with that of beets or asparagus.

The series of plants have been broadly classified into three groups by designating those whose relative-tolerance values were below 50 as *boron-sensitive plants*, those whose relative-tolerance values were between 50 and 100 as *boron-semitolerant plants*, and those whose relative tolerance values were above 100 as *boron-tolerant plants*.

The notable differences in the boron-tolerance values of different plants of the series are shown graphically in figure 3. In general, the growth depressions that resulted with increasing concentrations of boron in the substrate tend to be linear. The plants illustrated in figure 3 were chosen to show the marked differences in the slopes of the growth-depression curves.

BORON ACCUMULATION

EFFECT OF BORON CONCENTRATION IN NUTRIENT SOLUTION

The data presented in table 1 show outstanding differences in the boron-accumulation characteristics of the different species compared. The concentrations in the leaves of plants growing on the 5-boron solution ranged from 58 to 1,804 p. p. m. and in 25-boron from 209 to 3,875 p. p. m. The roots, stems, and fruits customarily contained

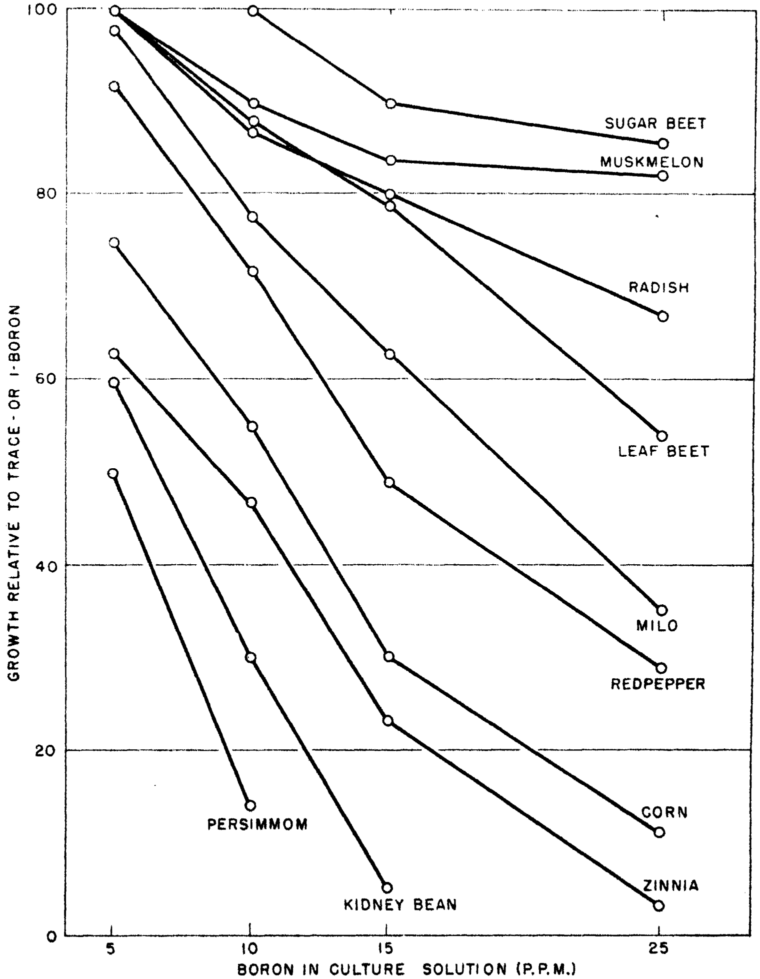


FIGURE 3.—Effect of boron on growth of plants, showing tendency toward linear relations between boron toxicity and the boron content of the substrate.

only a little boron as compared with that found in the leaves, but to this generalization peach and cherry are important exceptions.

The boron concentrations in the leaves of plants on trace-boron amounted (on dry-weight basis) to 100 to 5,000 times the concentration of boron in the nutrient solution; ratios between 400 and 1,200 predominated. On the 1-boron culture the accumulation ratios, on the same basis, ranged from 22 to 519, values between 50 and 200 predominating. In nearly all instances the accumulation ratios were substantially lower on 5- to 25-boron than on 1-boron, and many

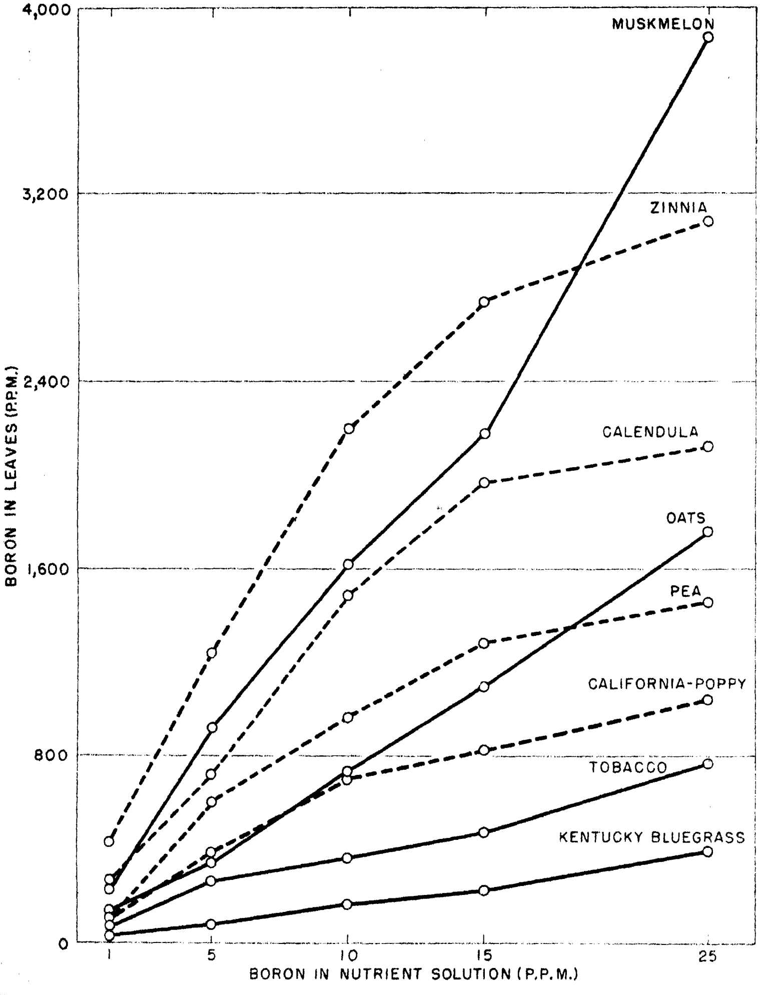


FIGURE 4.—Accumulation of boron in leaves of plants on solutions with 1 to 25 p. p. m. of boron. Solid lines=plants with linear accumulation ratios; broken lines=plants with decreasing ratios.

plants showed little change in their accumulation ratios between 5 and 25 p. p. m.

Between 5- and 25-boron, the series of plants tend to fall into one or the other of two groups: (1) Those with accumulation ratios that changed little (fig. 4) as the solution concentrations increased (accumulation linear) and (2) those that had well-defined decreasing ratios with the successively higher solution concentrations. Of the full series of plants, about as many tend to fall into one group as the other, but there are also a good many plants whose accumulation characteristics in the 5- to 25-boron range are not well defined by the data. The position of plants in one or the other group bears little relation either to the position of the plants as listed in order of tolerance or to position in terms of boron accumulation from the 5-boron solution.

Effect of Climatic Conditions

A general one-way relationship between climatic conditions and the extent of boron accumulation apparently does not exist. Of those plants included in both summer and winter planting, the onion and alfalfa accumulated more boron during the short-day cooler months than during the summer, whereas the opposite relationship was found in carrots, bluegrass, radishes, beets, and sweetclover.

Tolerance and Boron Accumulation

The correlation, as indicated by the data, between boron toxicity and the extent to which different species accumulate boron from a given substrate is apparently quite low. This fact is illustrated graphically by the scatter diagram (fig. 5), wherein boron concentrations in the leaves or laminae of the 5-boron plants are plotted against the relative-tolerance values.

MOVEMENT OF BORON IN PLANTS

Distribution of Boron in Different Parts of Plants

With the stone fruits as known exceptions (*8*), boron tends to accumulate in highest concentrations in the leaves of plants and to be present in relatively low concentrations in roots, wood, fruit, and other storage and meristematic tissues. In other words, having been carried into the leaves, presumably in the transpiration stream, it seems that there is a comparatively limited re-movement of boron. An examination of the distribution of boron in lemon leaves makes it evident, furthermore, that boron moves from the veins into the interveinal leaf tissues and toward the leaf margins and that there is comparatively little back movement through the mesophyll. The combined midveins and petioles, the green, the yellow, and the dead marginal portions of lemon leaves were found in one examination to contain respectively 47, 438, 1,060, and 1,722 p. p. m. of boron on the dry-weight basis, a distribution of boron that corresponds with the pattern of injury (pl. 1, *A*). A similar distribution has been found in walnut leaves (*8*), and the pattern of injury in many other plants suggests that this situation may be fairly general. Boron, on the other hand, is quite freely translocated from one region to another in the stone fruits, and it is probable that intermediate degrees of boron movement take place in other plants.

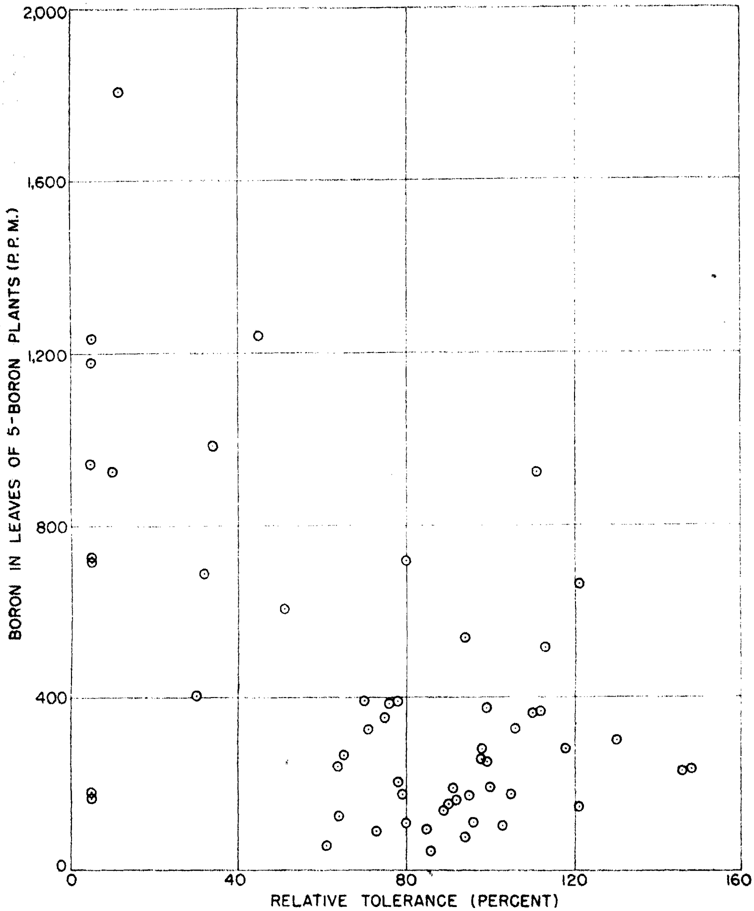


FIGURE 5.—Accumulation of boron in leaves of plants grown in 5-boron solution and the relative tolerance of these plants.

SOLUBILITY OF BORON IN LEAF TISSUES

The evidence of only a limited re-movement of boron from the leaves of many plants presents a question as to whether, after entering the mesophyll from the transpiration stream, boron is precipitated in the form of compounds of low solubility or whether it is combined in soluble molecules of such size or character that they are unable to re-pass the plasma membranes for movement to the other parts of the plant. The measurements presented in table 2 were made for the purpose of examining this question by comparing the concentrations of boron found by analysis in the expressed saps of a series of plants with the concentrations of boron calculated as possible in the same saps on the basis of the sap content of fresh leaves and analyses of the dried leaves.

If much or all of the total boron known to be present in leaves was found in the press cake, one would conclude that it had probably existed in the leaf in an insoluble form. If, on the other hand, the boron concentrations found in the sap expressed from leaves accounted for all or nearly all of the total boron, then it would be logical to conclude that much of it had been present in the sap in the form of soluble but immobile molecules.

TABLE 2.—*Concentrations of boron in expressed leaf sap and the concentrations indicated as possible on the basis of analyses of the dry leaves*

PLANTS WITH LITTLE OR NO PRECIPITATED BORON

Plant, date cropped, and boron concentration (p. p. m.) of nutrient solution	Boron in— Dry leaves (by analysis)	Boron in— Leaf sap: Calculated as possible	Boron in— Leaf sap: Found by analysis
	P. p. m.	*P. p. m.*	*P. p. m.*
Alfalfa (May 4, 1933):			
Trace	30	4	(1)
1	199	32	31
5	272	46	38
10	343	60	54
15	531	94	115
25	822	147	176
Sugar beet (crop 68):			
Trace	19	2	2
1	106	9	7
5	234	21	20
10	495	44	46
15	588	51	51
25	975	83	95
Calendula (Aug. 14, 1933):			
Trace	54	3	4
1	231	13	----------
5	604	38	34
10	1, 226	79	91
15	1, 726	114	111
25	2, 298	189	184
Cowpea (crop 10):			
Trace	32	3	(1)
1	119	13	6
5	404	45	50
10	722	78	86
15	1, 132	----------	----------
25	----------	----------	----------
Kentucky bluegrass (crop 31, May):			
Trace	3	1	(1)
1	22	6	2
5	92	27	17
10	164	40	42
15	224	65	61
25	398	----------	----------
Lettuce, Los Angeles Market (crop 50):			
Trace	27	1	(1)
1	70	2	(1)
5	116	4	2
10	221	7	8
15	343	13	12
25	582	25	29
Lettuce, Big Boston (crop 51):			
Trace	43	2	3
1	75	2	2
5	166	7	7
10	353	14	12
15	459	23	24
25	817	46	46
Kidney bean (crop 15):			
Trace	(1)	(1)	3
1	151	20	20
5	690	91	83
10	1, 254	201	190
15	1, 733	----------	----------
25	----------	----------	----------

[1] Trace.

TABLE 2.—*Concentrations of boron in expressed leaf sap and the concentrations indicated as possible on the basis of analyses of the dry leaves*—Continued

PLANTS WITH LITTLE OR NO PRECIPITATED BORON—Continued

Plant, date cropped, and boron concentration (p. p. m.) of nutrient solution	Boron in—		
	Dry leaves (by analysis)	Leaf sap	
		Calculated as possible	Found by analysis
	P. p. m.	*P. p. m.*	*P. p. m.*
Pea (crop 21):			
Trace	6	2	(1)
1	212	33	31
5	609	118	116
10			
15	1,283		
25	1,452		
Tobacco (crop 52):			
Trace	19	2	1
1	72	5	5
5	261	20	16
10	365	26	28
15	474	35	45
25	771	54	56
Turnip (May 3, 1933):			
Trace	22	2	(1)
1	84	6	3
5	189	13	9
10	258	22	14
15	446	34	31
25	744	64	68

PLANTS WITH MUCH PRECIPITATED BORON

Plant, date cropped, and boron concentration (p. p. m.) of nutrient solution	Dry leaves (by analysis)	Leaf sap: Calculated as possible	Leaf sap: Found by analysis
Muskmelon (crop 61):[2]			
Trace	30	3	1
1	234	20	13
5	1,010	92	37
10	1,835	151	78
15	2,249	204	109
25	3,836	323	191
Sweetpotato (Aug. 15, 1933):			
Trace	57	5	(1)
1	152		
5	706	88	49
10	1,301	138	108
15	1,646		
25	2,506		
Tomato (crop 55):			
Trace	34	3	1
1	103	9	4
5	253	23	19
10	531	51	26
15	684	61	34
25	1,168	133	82

[1] Trace.
[2] Boron in green leaves (dry basis). The weighted means of green and dead leaves were reported in table 1.

Aliquots of freshly picked leaves, packed in well-stoppered, long, glass test tubes, were frozen in solid carbon dioxide overnight; the material was then quickly thawed and the sap expressed as rapidly as possible in a Carver press at 2,400 pounds' pressure per square inch. The "possible" concentration of boron in the leaf sap was computed by dividing the oven-dry weight of the press cake by the weight of sap (difference in weight of the fresh sample and oven-dry press cake) and multiplying the quotient by the parts per million of boron found in a dried aliquot of the original leaf material. Some uncertainty must accompany estimates of this character both as regards changes in solubility during freezing and pressing and as regards the proportion of the leaf water that functions as a solute in living tissue. It seems doubtful, nevertheless, that the issue here under examination could be wholly confused by these considerations.

The data of table 2 indicate the existence of 2 classes of plants: (1) Those having little or no insoluble boron in their leaves, and (2) those having substantial quantities of insoluble or slightly soluble boron. Of the 14 kinds, 11 fell in the former class and 3 in the latter. In each of the latter 3 kinds (muskmelon, sweetpotato, and tomato) 50 percent of the boron is indicated as having been insoluble or fixed. Notwithstanding this evidence of some insolubility in the latter 3 kinds, the concentrations of soluble boron in all the expressed leaf saps were so high that the low concentrations in the stem and root tissues cannot be explained on the basis of insolubility. Any theory advanced to account for the immobility of the boron in leaves that assumes precipitation with organic or inorganic constituents of the leaf cells would require, if valid, that essentially all of the boron in the leaves should be insoluble. In none of these plants was that found to be the case, yet in all of them the boron concentrations in tissues other than the leaves were by comparison relatively low (table 1). It is most logical to conclude, therefore, that the low mobility of boron after having entered the leaf mesophyll is due not to insolubility but instead to some inability of the soluble molecule of which the boron evidently becomes a part to move out of the cells.

It is well known that boron unites freely with a number of sugar-like substances. The fact that before reaching the leaf mesophyll boron, like other inorganic constituents, must pass through living cells of the root implies that the reaction taking place in leaves may be either photochemical or one that takes place with photosynthetic products occurring in higher concentrations in leaves than elsewhere. Other observations (table 1) likewise indicate that light is in some manner a factor that limits boron movement.

The concentration of boron in the roots of onions, radishes, and carrots was higher, relative to that in the leaves, when these plants were grown as winter crops than as summer crops. The bulbs of onions cropped from the 25-boron bed in April (table 1, crop 25) contained three times as high a concentration of boron as those cropped in July (crop 26), and yet the concentrations of boron in the leaves of the two crops were similar. The roots of 25-boron radishes (crops 39 to 42) cropped in February, November, March, and July had concentrations respectively 47, 31, 24, and 16 percent as high as the leaves, and the corresponding average daily hours of sunshine were 6.4, 7.8, 8.0, and 12.1. Carrots cropped from the 25-boron bed in April (crop 28) and September (crop 29) had respectively 788 and 1,244 p. p. m. of boron in their leaves and 319 and 114 p. p. m. in their roots; the average hours of sunshine per day for the carrots were 9.5 and 11.0 respectively. In all of these plants retention of boron in leaves increased as hours of sunshine increased. Beets did not accumulate much boron in their roots in any planting, and the differences between summer and winter plantings are of doubtful significance.

As shown by the foregoing and the example provided by the stone fruits, there is evidently a wide difference between plants in the extent of boron movement from leaves to other parts. Attention has been called previously (7) to a 40 percent greater accumulation of boron in leaves of sunflower on basal branches of plants with grafted-in

Jerusalem-artichoke tops than in similarly situated sunflower leaves on plants without Jerusalem-artichoke tops. This finding was thought to mean that less boron was fixed in Jerusalem-artichoke than in sunflower leaves and that a movement occurred from the former to the latter.

Boron Movement and Boron Deficiency

Little has been said in this paper about relations between the severity of boron-deficiency manifestations and the concentrations of boron found by analysis in the leaves of the plants. The omission is occasioned in part by the possibility that many of the variations shown in the boron analyses of the trace-boron plants are within the range of the analytical error for plant material. On the other hand, good correlations might not have been found in this borderline, or marginal, range even if the leaf analyses had all been highly accurate. The marked differences between plants in the ratios of boron in leaves to boron in other parts, the differences in accumulation in roots between winter and summer plantings, and the variations in the severity of deficiency symptoms in winter and in summer all point to the importance of the boron-movement factor. The importance of this factor is further illustrated by the observation of a number of investigators that deficiency symptoms appear quite soon after plants are transferred from plus- to minus-boron solutions. The observation undoubtedly means that little of the boron present in the older leaves is moved into the developing meristematic tissues. In other words, the boron concentration found in leaf tissues does not necessarily provide a criterion of the concentrations present in the actively growing tissues of the plant and for normal growth the boron evidently must be present in the tissue concerned. Within the marginal ranges of soil boron concentrations, factors influencing the movement of boron into meristematic tissues from leaves may thus be almost as important in terms of boron deficiency as the boron-supplying power of the soil itself.

There is no evident reason for believing that the movement of boron into terminal buds or other growth tissues that utilize materials translocated from leaves is conditioned by factors very different from those determining the movement of boron from leaves to the roots. The data relating to such translocation may appropriately be examined for parallelisms between climatic factors and the accumulation of boron in root tissues and between climatic factors and boron deficiency.

Plants grown both in summer and winter had less boron in their roots, relative to that in their leaves, in summer crops than in winter crops, and the summer crops tended to show the most marked manifestations of boron deficiency. More marked symptoms of deficiency developed during the relatively bright summer of 1929 than during subsequent summers. Radishes, like other plants, had more boron in their roots, relative to the concentrations in the leaves, in winter than in summer; yet, in contrast with other plants, the deficiency symptoms were most marked in the winter. This apparent discrepancy in the relation between high light intensity, restricted boron movement, and deficiency symptoms is clarified when attention is directed to the fact that the symptoms of boron deficiency exhibited by the radish were confined to the root tissues and in actual concentration there was less boron in the radish roots in the winter than in summer.

There is a widely prevalent and apparently well supported idea that boron deficiency is more pronounced in dry summers than in rainy ones. Inasmuch as dry summers are usually characterized by a predominance of bright days, the possibility exists that the apparent relationship between moisture conditions and boron deficiency is more truly a relationship between average light intensity and boron deficiency. The data presented in this paper are not sufficient to warrant the definite conclusion that light intensity is a dominant factor determining the extent of boron deficiency when plants are grown with a marginal supply of boron during dry seasons, but the limited evidence points in that direction. Long before boron deficiency was recognized as a cause of the drought spot and related diseases of apples, Mix (*9*) concluded that these diseases were "greatly influenced if not caused by lack of sufficient moisture, but insufficient moisture cannot be looked upon as the sole cause." A similar conclusion has been reached by a number of subsequent investigators of these disorders of the apple. Walker and his associates (*12*) have likewise reported that boron deficiency in beets is most pronounced in dry summers.

Drying has been found to be an important adjunct to boron fixation when new boron is added to a soil, but there is as yet no evidence that drying reduces the boron concentration of the soil solution when new boron is not added. On the contrary the concentration of boron in the soil solution has been found to be higher at the moisture equivalent than at higher moisture percentages. When soils without added boron were dried and then rewetted the initial boron concentrations were again found.

SUMMARY

For the purpose of obtaining comparative data on symptoms, growth reactions, and boron-accumulation characteristics of different plants, 50 species (58 varieties) were grown in each of 6 large outdoor sand cultures supplied respectively with a trace (0.03 to 0.04 p. p. m.), 1, 5, 10, 15, and 25 p. p. m. of boron. The experiments were conducted at Riverside, Calif., during the period May 1930 to March 1934.

Approximately 25 percent of the plants made their greatest growth in the trace-boron culture, and the others responded to 1 p. p. m. or more of boron; the growth of a number of the species was increased by boron concentrations as high as 10 and 15 p. p. m.

Of the 58 varieties, 20 developed morphological symptoms of boron deficiency when grown in trace-boron, and the maturity of 4 varieties was markedly affected; 4 plants were more subject to mildew in trace-boron than in the higher concentrations.

The data provide a substantial basis for the conclusion that there is considerable overlapping between the injurious and the beneficial effects of boron within plants, inasmuch as mild to marked leaf injury was observed in 19 of the 72 plants (including repetitions) at or below the substrate concentrations that resulted in greatest growth.

The concentrations of boron in the leaves of plants (dry-weight basis) growing in 5-boron ranged from 58 to 1,804 p. p. m. and in 25-

boron from 209 to 3,875 p. p. m. The concentrations of boron in roots, stems, and fruit were generally much lower than those in the leaves; the concentrations of boron in entire plants tended to average about half of that in the leaves.

Numerical values for the tolerance of the plants to boron were derived by dividing the average of the dry weights in 5-, 10-, and 15-boron by the weight in trace- or 1-boron (whichever was higher) and multiplying this quotient by 100. These values ranged from below 10 for the highly sensitive plants to above 200 for one of the most tolerant plants.

Ratios of the concentration of boron in leaves (dry-weight basis) to boron in the trace-boron nutrient solution were usually between 400 and 1,200 and in 1-boron between 50 and 200. Lower ratios were found if the boron concentrations of the substrates were higher.

Little relation was found between boron tolerance and the boron-accumulation characteristics of different species.

Boron after entering the leaf tissues of most plants tends to remain there instead of being moved freely out, with sugars and other compounds, to growing tissues. The translocation of boron to the bark and wood and subsequently to the flesh of fruit takes place readily, however, in the stone fruits.

Comparisons between the boron concentrations in the sap expressed from frozen leaves and that estimated as possible in the sap on the basis of the moisture content and analyses of entire leaves indicated that all or nearly all of it was in solution in the leaves of 11 of the kinds tested and about half of it in 3 kinds. The accumulation of boron in leaves appears to be due to the formation of compounds with large or otherwise immobile molecules that are unable to diffuse through the plasma membranes.

A number of plantings grown during the winter months were repeated in summer. Higher concentrations of boron were found in winter-grown alfalfa and onions; but carrots, bluegrass, radishes, beets, and sweetclover accumulated more boron when grown in the summer.

Onions, radishes, and carrots accumulated several times as much boron in their roots, relative to the concentrations in the leaves, when grown in winter as when grown in summer. High light intensity and possibly the higher temperatures were seemingly unfavorable during the summer to the movement of boron from the leaves to other organs of these plants. The data suggest that boron after entering the mesophyll reacts with leaf-cell constituents, forming soluble but relatively immobile compounds. The extent to which boron is immobilized is evidently related to the intensity or to the average duration of light.

Boron deficiency of a number of the plants was most pronounced under the climatic conditions that restricted the movement of boron from the leaves to other tissues. The results indicate that factors affecting the movement and distribution of boron in the plant may be almost as important in determining boron requirements as the boron-supplying power of the substrate.

LITERATURE CITED

(1) Brandenburg, E.
1931. DIE HERZ- UND TROCKENFÄULE DER RÜBEN ALS BORMANGELERSCHEINUNG. Phytopath. Ztschr. 3: 499–517, illus.

(2) Brenchley, W. E., and Warington, K.
1927. THE ROLE OF BORON IN THE GROWTH OF PLANTS. Ann. Bot. [London] 41: 167–187, illus.

(3) Christensen, J. J.
1934. NONPARASITIC LEAF SPOTS OF BARLEY. Phytopathology 24: 726–742, illus.

(4) Eaton, F. M.
1930. THE EFFECT OF BORON ON POWDERY MILDEW AND SPOT BLOTCH OF BARLEY. Phytopathology 20: 967–972, illus.

(5) ———
1931. A LARGE SAND CULTURE APPARATUS. Soil Sci. 31: 235–240, illus.

(6) ———
1940. INTERRELATIONS IN THE EFFECTS OF BORON AND INDOLEACETIC ACID ON PLANT GROWTH. Bot. Gaz. 101: 700–705, illus.

(7) ——— and Blair, G. Y.
1935. ACCUMULATION OF BORON BY RECIPROCALLY GRAFTED PLANTS. Plant Physiol. 10: 411–424, illus.

(8) ——— McCallum, R. D., and Mayhugh, M. S.
1941. QUALITY OF IRRIGATION WATERS OF THE HOLLISTER AREA OF CALIFORNIA WITH SPECIAL REFERENCE TO BORON CONTENT AND ITS EFFECT ON APRICOTS AND PRUNES. U. S. Dept. Agr. Tech. Bul. 746, 59 pp., illus.

(9) Mix, A. J.
1916. CORK, DROUTH SPOT AND RELATED DISEASES OF THE APPLE. N. Y. State Agr. Expt. Sta. Bul. 426: 473–522, illus.

(10) Morris, H. S.
1931. PHYSIOLOGICAL EFFECTS OF BORON ON WHEAT. Torrey Bot. Club Bul. 58: 1–30, illus.

(11) Sommer, A. L.
1927. THE SEARCH FOR ELEMENTS ESSENTIAL IN ONLY SMALL AMOUNTS FOR PLANT GROWTH. Science 66: 482–484.

(12) Walker, J. C., Jolivette, J. P., and McLean, J. G.
1938. INTERNAL BLACK SPOT OF CANNING BEETS AND ITS CONTROL. Canning Age 19: 489 [491], 508, illus.

(13) Warington, K.
1933. THE INFLUENCE OF LENGTH OF DAY ON THE RESPONSE OF PLANTS TO BORON. Ann. Bot. [London] 47: 329–457, illus.

(14) Wilcox, L. V.
1930. DETERMINATION OF BORON IN NATURAL WATERS AND PLANT MATERIALS. Indus. and Engin. Chem., Analyt. Ed. 2: 358–361, illus.

(15) ———
1932. ELECTROMETRIC TITRATION OF BORIC ACID. Indus. and Engin. Chem., Analyt. Ed. 4: 38–39.

(16) Yarwood, C. E.
1938. THE EFFECT OF BORON NUTRITION ON THE SUSCEPTIBILITY OF SOME PLANTS TO POWDERY MILDEWS. (Abstract) Phytopathology 28: 22.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., OCTOBER 1, 1944 No. 7

FACTORS INFLUENCING EMBRYONATION AND SURVIVAL OF EGGS OF THE STOMACH WORM, HAEMONCHUS CONTORTUS [1]

By DOYS A. SHORB

Associate parasitologist, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The large stomach worm, *Haemonchus contortus*, is in many respects the most serious parasite of ruminants. It occurs in the fourth stomach, or abomasum. The adult worms produce large numbers of eggs, which are in the morula stage when they are discharged with the feces of the host. Under favorable conditions the eggs develop to the tadpole stage in 8 hours or less and to the vermiform embryo stage in 24 hours. Shortly after this the eggs hatch. The first- and second-stage larvae each have a life span lasting a little more than a day under favorable conditions. The third, or infective larvae, may be produced, therefore, on the fourth or fifth day after the eggs are passed out of the host animal.

The control of these roundworms depends on a thorough knowledge of the free-living stages and of the factors that influence their development and survival. The work reported in this paper was undertaken to test the effects of certain adverse environmental conditions on the embryonation and survival of the eggs, one of the free-living stages of this parasite. The investigations were carried out at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., from August 1936 to February 1940.

MATERIALS AND METHODS

Feces of sheep experimentally infected with *Haemonchus contortus* were used as source material for the experiments. Freedom of the material from other parasites was tested by examinations of feces for eggs (*10*) [2], examination of fecal cultures for infective larvae, and post mortem examinations of the gastrointestinal tracts of some of the host animals that had served as a source of eggs. The only other nematode found was *Strongyloides papillosus*. For purposes of the present investigation, the presence of *S. papillosus* was not a complicating factor because this nematode is readily differentiated in all its stages from the corresponding stages of *H. contortus*.

Unless otherwise stated, all cultures were made from 24-hour fecal samples collected in muslin bags attached to rams or wethers and, therefore, not contaminated with urine. The fecal pellets were pulverized and the resulting mass thoroughly mixed, because it had been previously determined that more larvae developed in feces treated in this manner than when the unbroken pellets were cultured.

---

[1] Received for publication February 13, 1943.

[2] Numbers in parentheses refer to Literature Cited, p. 286.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 7
Oct. 1, 1944
Key No. A-214

610084—44——1

larvae, but in view of the results of the present experiment it appears probable that infective larvae rather than eggs were present.

In view of the fact that there is a marked reduction in the number of larvae developing in cultures under reduced oxygen conditions, it appears that only a few larvae of *H. contortus* would develop in sheep feces dropped or washed into ponds or other bodies of water.

EFFECT OF HIGH AND LOW TEMPERATURES

Veglia (*14*) reported that *Haemonchus contortus* eggs embryonated normally at temperatures between 68° and 95° F., which he considered to be optimum. The following experiments were performed to determine the effect on the eggs of exposure to less favorable temperatures.

In these experiments pint-jar cultures, after they had been exposed to unfavorable temperatures in incubators and electric refrigerators, were maintained at room temperature for a period sufficient to allow

TABLE 2.—*Effect of exposure to low and high temperatures on survival of eggs of Haemonchus contortus*

Experiment No.	Temperature	Exposed culture No.	Time of exposure	Infective larvae recovered from—	
				Exposed cultures	Control cultures[1]
	°F.		*Days*	*Number*	*Average number*
1	10	1	1	2	7,562
		2	2	0	
		3	3	0	
2	15	1	2	280	3,781
		2	2	0	
3	25	1	2	14	14,310
		2	2	44	
4	33	1	1	9,280	40,260
5	33	1	2	330	14,310
		2	2	400	
6	33-39	1	1	1,222	7,562
		2	2	928	
		3	3	370	
		4	4	39	
		5	5	39	
		6	6	63	
		7	9	0	
7	36	1	1	1,234	3,781
		2	2	84	
8	37-45	1	2	3,478	7,562
		2	3	1,404	
9	39-49	1	14	24	10,710
		2	28	0	
10	40	1	1	33,840	40,260
11	40-52	1	14	63	10,710
		2	28	1	
12	45-55	1	14	123	10,710
		2	28	11	
13	90-96	1	2	5,160	7,562
		2	3	7,380	
		3	4	6,480	
		4	5	8,960	
14	93-99	1	2	8,000	7,562
		2	3	9,890	
		3	4	8,940	
		4	5	8,029	
15	98	1	1	2,320	3,781
		2	2	1,634	
16	98	1	1	7,875	14,310
		2	2	2,400	
			Hours		
17	140	1	0.5	5,560	10,710
		2	1	2,244	
		3	1.5	1	
		4	2	2	
		5	2.5	0	
		6	3	0	

[1] Exposed at room temperature—about 70° F.

any surviving eggs to hatch and for the larvae to reach the infective stage. The results of 17 experiments are shown in table 2.

The data in this table show that any marked deviation from the temperatures found by Veglia to be optimum caused a drop in the number of larvae reaching the infective stage or of eggs capable of reaching the infective stage when subjected to favorable conditions. These data also give some new information on the rapidity of devitalization of eggs of *H. contortus*, namely, that few eggs remained viable for 1 or 2 days at temperatures below freezing (experiments 1, 2, and 3), and that in 1-day exposures to temperatures just above freezing (experiments 4, 6, and 7) two-thirds to five-sixths of the eggs became devitalized. The data are especially important in showing that few eggs of *H. contortus* are able to survive as long as 2 weeks at temperatures only 12° to 28° F. below those considered optimum for embryonation (experiments, 9, 11, and 12).

Veglia (*14*) found that some second-stage larvae developed from eggs kept at temperatures as low as 46.4° F. Observations not included in table 2 indicated that no development of larvae to infectivity took place in cultures kept at temperatures below 55°, although a few eggs survived at these temperatures for as long as 4 weeks, as shown in experiments 11 and 12 of table 2. From this information, as well as from the data shown in table 2 that only 2 to 12 out of 1,000 eggs survived temperatures of 39° to 55° for 2 weeks, and only about 1 out of 1,000 survived at 55° for 4 weeks, it may be inferred that *H. contortus* eggs in feces deposited on pasture will not yield larvae capable of reaching infectivity while the weather is cold.

To determine the effect of outdoor temperatures during December on the survival of eggs of *H. contortus*, nine 4-inch Petri-dish cultures each containing 20 gm. of feces, were prepared. Seven of these were placed outside in an experimental plot, and two were retained in the laboratory as controls. The cultures placed outdoors were covered with opaque paper and were removed to the laboratory one at a time. During the 9 days that the cultures were outdoors the temperature fell below freezing four times, once on each of the first 4 days of the experiment. The lowest temperature recorded, 17° F., occurred during the second night of the experiment. All cultures were examined 6 days after the last culture had been brought into the laboratory. The results are recorded in table 3.

TABLE 3.—*Survival of eggs of Haemonchus contortus exposed to outdoor temperatures in December*

Culture No.	Outdoor temperatures		Time of exposure	Total period each culture was below freezing	Larvae recovered [1]
	Minimum	Maximum			
	°F.	*°F.*	*Days*	*Hours*	*Number*
1	28	37	1	6	46
2	17	39	2	22	0
3	17	50	3	36	10
4	17	60	5	46	0
5	17	63	7	46	4
6	17	63	8	46	0
7	17	63	9	46	0
8 [1]					120
9 [1]					220

[1] Control cultures.

of the sheep and lambs raised in this country are found where the winters are cold and embryonation of eggs in normal years would be possible only from May to September, inclusive. Although the experiments involving the effect of reduced oxygen supply on the survival of *H. contortus* eggs may be only of theoretical importance so far as field conditions are concerned, they indicate that as a source of *Haemonchus* infection swamps and ponds with much organic matter may have been overrated. Most *H. contortus* eggs falling in such ponds or on swampy ground would die for lack of sufficient oxygen.

SUMMARY AND CONCLUSIONS

To test the effect of lack of oxygen, drying, and adverse temperatures on the survival of eggs of the large stomach worm, *Haemonchus contortus*, investigations were carried out at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., from August 1936 to February 1940. Feces of sheep experimentally infected with *H. contortus* were used as source material for the experiments.

After culture jars were sealed so that no fresh air could enter, embryonation of *H. contortus* eggs in the cultures ceased, and in less than 4 weeks but more than 3 weeks the viability of the eggs was destroyed.

At temperatures below 55° F. no development to infectivity of *H. contortus* took place, and death resulted if the exposure was sufficiently long. Constant temperatures above 98° were deleterious to the eggs. There was little difference in the number of larvae that developed from eggs kept at temperatures of 90° to 96° and 93° to 99° and those kept at 70°. The lethal effect observed increased with the deviation from the optimum temperatures.

Drying killed eggs rapidly, but a few survived several hours in apparently dry feces. When the moisture content of the feces was lowered by drying the feces at a relatively low humidity or by means of air currents at a relatively high humidity, the death rate of eggs was raised.

LITERATURE CITED

(1) Boughton, I. B., and Hardy, W. T.
1938. STOMACH WORMS (HAEMONCHUS CONTORTUS) IN SHEEP AND GOATS. Tex. Agr. Expt. Sta. Ann. Rpt. 51: 217–218.

(2) Boycott, A. E., and Haldane, J. S.
1904. "ANKYLOSTOMIASIS," NO. II. Jour. Hyg. [London] 4: 73–111.

(3) Johnson, E. L.
1924. RELATION OF SHEEP TO CLIMATE. Jour. Agr. Res. 29: 491–500.

(4) Leiper, R. T.
1929. THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE SPREAD OF PARASITIC WORMS. Agr. Met. Conf. Rpt. (So. Kensington, England) (1929): 79–82.

(5) Lerche, (M.)
1929. UEBER DIE ENTWICKLUNGSBEDINGUNGEN UND ÜBER DIE BEKÄMPFUNGSMÖGLICHKEIT DES ROTWURMES (SYNGAMUS TRACHEALIS) BEIM GEFLÜGEL. Arch. f. Geflügelk. 3: 24–27.

(6) Mönnig, H. O.
1930. STUDIES ON THE BIONOMICS OF THE FREE-LIVING STAGES OF TRICHOSTRONGYLUS SPP. AND OTHER PARASITIC NEMATODES. Union So. Africa Dept. Agr. Rpt. Dir. Vet. Res. 16: 175–198.

(7) Parona, C., and Grassi, B.
1878. SULLO SVILLUPO DELL'ANCHILOSTOMA DUODENALE ANNOTAZIONI. Soc. Ital. di Sci. Nat. 21: 53–58.

(8) PERRONCITO, E.
1880. OSSERVAZIONI ELMINTOLOGICHE RELATIVE ALLA MALLATTIA SVILUPPATASI ENDEMICA NEGLI OPERAI DEL GOTTARDO. Atti r. Acad. Lincei, Mem. Sci. Fis., Mat. e Nat. (1879–80) (3) 7: 381–433.

(9) POLUSZYNSKI, G.
1930. MORPHOLOGISCH-BIOLOGISCHE UNTERSUCHUNGEN ÜBER DIE FREILEBENDEN LARVEN EINIGER PFERDESTRONGYLIDEN. Tierärztl. Rundschau 36: 871–873, illus.

(10) SHORB, D. A.
1939. DIFFERENTIATION OF EGGS OF VARIOUS GENERA OF NEMATODES PARASITIC IN DOMESTIC RUMINANTS IN THE UNITED STATES. U. S. Dept. Agr. Tech. Bul. 694, 10 pp., illus.

(11) SKRIABINE, K. I., and SHULTZ, R. E. S.
1936. LES HELMINTHOSES PULMONAIRES DES ANIMAUX. Off. Internatl. des Épizoot. Bul. 12: 407–456, illus.

(12) SPINDLER, L. A.
1931. VIABILITY OF EGGS AND LARVAE OF STEPHANURUS DENTATUS. Jour. Parasitol. 18: 45–46.

(13) THEILER, A., and ROBERTSON, W.
1915. INVESTIGATIONS INTO THE LIFE-HISTORY OF THE WIRE-WORM IN OSTRICHES. Union So. Africa Dept. Agr. Rpt. Dir. Vet. Res. 3 and 4: 291–345.

(14) VEGLIA, F.
1915. THE ANATOMY AND LIFE HISTORY OF THE HAEMONCHUS CONTORTUS (RUD.) Union So. Africa Dept. Agr. Rpt. Dir. Vet. Res. 3 and 4: 347–500.

STUDIES ON THE USE OF THE POINT-QUADRAT METHOD OF BOTANICAL ANALYSIS OF MIXED PASTURE VEGETATION[1]

By William B. Drew[2]

Assistant professor, Department of Botany, Missouri Agricultural Experiment Station

INTRODUCTION

Since the development of the point-quadrat method for the analysis of pasture vegetation originated by Levy and Madden[3] in New Zealand, there have appeared in the United States a number of studies on its use and applications. Following an intensive comparative investigation in Wisconsin of several methods of botanical analysis, Tinney, Aamodt, and Ahlgren[4] concluded that the point-quadrat method gave the greatest promise of yielding reliable data consistent with rapidity and ease of operation. Hanson[5] had earlier found that the method was reliable when employed in analytical work on the native prairies of western North Dakota. More recently, Arny and Schmid,[6] working with various pasture mixtures in Minnesota, have investigated critically the use of the inclined point-quadrat method. These investigators showed that in mixtures of tall legumes and fine-leaved grasses correction factors must be computed in order precisely to evaluate the data, since the broad-leaved legumes tended to be hit less per gram of dry weight than the narrow-leaved grasses.

The present study was undertaken in order to determine (1) the relative merits of the point-quadrat method as compared with the count-list method for sampling small areas of a low-growing, grass-lespedeza pasture; and (2) the relative efficiency of four different point-quadrat methods for the quantitative analysis of such vegetation.

The investigation was carried out during July and August 1942 on a lespedeza-small grain rotation pasture in the vicinity of Columbia, Mo. Because of unfavorable planting conditions in the fall of 1941, it was impossible to sow the small grain successfully. Hence, the next spring and summer the pasture had an astonishingly uniform cover of the palatable fall panicum, (*Panicum dichotomiflorum*), which had replaced the grain, in addition to an excellent stand of lespedeza (*Lespedeza stipulacea*). While the fall panicum is hardly in the usual

---

[1] Received for publication April 6, 1943. Contribution from the Department of Botany, Missouri Agricultural Experiment Station, Journal Series No. 890.

[2] The writer wishes to express his appreciation to Dr. W. C. Etheridge, professor and chairman, and Dr. E. M. Brown, assistant professor, of the Department of Field Crops, University of Missouri, for their cooperation in facilitating this study; and to Profs. A. C. Arny and A. R. Schmid, Division of Agronomy and Plant Genetics, University of Minnesota, for a number of helpful suggestions.

[3] Levy, E. B., and Madden, E. A. THE POINT METHOD OF PASTURE ANALYSIS. New Zeal. Jour. Agr. 46: 267-279, illus. 1933.

[4] Tinney, F. W., Aamodt, O. S., and Ahlgren, H. L. PRELIMINARY REPORT OF A STUDY ON METHODS USED IN BOTANICAL ANALYSES OF PASTURE SWARDS. Amer. Soc. Agron. Jour. 29: 835-840, illus. 1937.

[5] Hanson, H. C. A COMPARISON OF METHODS OF BOTANICAL ANALYSIS OF THE NATIVE PRAIRIE IN WESTERN NORTH DAKOTA. Jour. Agr. Res. 49: 815-842, illus. 1934.

[6] Arny, A. C. and Schmid, A. R. A STUDY OF THE INCLINED POINT-QUADRAT METHOD OF BOTANICAL ANALYSIS OF PASTURE MIXTURES. Amer. Soc. Agron. Jour. 34: 238-247. 1942.

Journal of Agricultural Research,
Washington, D. C

Vol. 69, No. 7
Oct. 1, 1944
Key No. Mo-14

category of forage crops, in its growth form under pasture conditions it closely resembles several domesticated grasses. Thus the results reported herein are thought to be reasonably applicable to lespedeza—grass mixtures in which the grass component is broader-leaved than Kentucky bluegrass.

PRELIMINARY COMPARISON OF THE POINT-QUADRAT, COUNT-LIST, AND WEIGHT-LIST METHODS OF ANALYSIS

PROCEDURE

An area supporting a relatively uniform mixed stand of grass and lespedeza, and having practically uniform soil and topographic conditions, was selected, and a plot of 100 m.^{2} (10×10 m.) was staked off. Ten one-half-square-meter quadrats were then selected at random. In all cases, the order of study was as follows: (1) Count-list; (2) inclined point-quadrat; (3) .straight point-quadrat; (4) weight-list. After careful counting of stems of individual plants [7] with a minimum of disturbance of their natural positions of growth, the point-quadrat apparatus was set up parallel to the long axis and across the half-square-meter sample area. Readings were first taken with the apparatus set up in the inclined (45°) position; and, then, without moving the two front supports, the apparatus was so placed that the needles were vertical. In each position, two types of data were recorded as follows: (1) The first species hit per needle (method A); and (2) the total number of hits obtained by pushing each needle to the ground, irrespective of whether the same plant or different ones were hit once or more than once (method B). Second and third sets of readings of 10 needles each were next obtained by resetting the apparatus in the quadrat, diagonally to the right, and then diagonally to the left. Afterwards, the entire one-half-square-meter area was clipped by hand at the soil surface, and, later, the species were hand-separated in the laboratory, the vegetation was oven-dried, and dry weights were obtained. For convenience, three components of the vegetation were separated, as follows: (1) Lespedeza; (2) grasses; (3) forbs.[8] Since other legumes were very rare, and since 98 percent of all grasses was fall panicum, no further separation appeared necessary. This part of the study was carried on only in early July.

RESULTS

The data in table 1 summarize the results of the count-list determinations. Although variation from quadrat to quadrat is evident, standard deviations and standard errors were not computed because the comparative magnitude of differences between methods is such as to indicate quite clearly these essential differences.

Comparing the percentage composition figures of table 1 with those of table 2, it is obvious that the use of the count-list method results in a decided underestimate of the amount of vegetation furnished by lespedeza, and vice versa, a noticeable overestimate of the grass element. Forbs were relatively scarce, and are not important enough to be worth further consideration at this time. So far as lespedeza is concerned, an undercount was certainly to be expected because of the widely branching nature of the plant. Similarly, the overcount of grasses further emphasizes the difficulties inherent in any direct counting of stems of individuals of such plants. The unit used for direct counting was the culm; but the point-quadrat data for grasses include hits on any aerial part of the plant.

---

[7] In the case of grasses, culms were taken as the unit for counting.

[8] The forbs included all broad-leaved herbs that were samples. The following species were the principal ones sampled: Bracted plantain (*Plantago aristata*), bullnettle (*Solanum carolinense*), cocklebur (*Xanthium* sp.), common ragweed (*Ambrosia artemisifolia* var. *elatior*), curly dock (*Rumex crispus*), field dodder (*Cuscuta pentagona*), knotweed (*Polygonum aviculare*), lanceleaf ragweed (*Ambrosia bidentata*), milk purslane (*Euphorbia supina*), Pennsylvania smartweed (*Polygonum pensylvanicum*), peppergrass (*Lepidium virginicum*), red sorrel (*Rumex acetosella*), spiny sida (*Sida spinosa*), yarrow (*Achillea millefolium*).

TABLE 1.—*Summary of results of count-list determinations*

Quadrat No.	Lespedeza		Grasses		Forbs	
	Plants	Composition	Plants	Composition	Plants	Composition
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	564	38. 6	886	60. 6	11	0. 8
2	313	20. 7	1, 115	73. 6	87	5. 7
3	388	31. 7	821	67. 0	16	1. 3
4	283	22. 9	920	74. 3	35	2. 8
5	364	30. 8	748	63. 2	71	6. 0
6	287	29. 3	670	68. 4	23	2. 3
7	367	42. 9	461	53. 9	27	3. 2
8	294	26. 4	771	72. 3	15	1. 3
9	269	27. 2	645	70. 3	25	2. 5
10	291	28. 8	625	69. 9	13	1. 3
Total	3, 420	299. 3	7, 662	673. 5	323	27. 2
Mean	342	29. 9	766. 2	67. 4	32. 3	2. 7

TABLE 2.—*Summary of results of weight-list determinations*

Quadrat No.	Lespedeza		Grasses		Forbs	
	Weight	Composition	Weight	Composition	Weight	Composition
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
1	28. 05	57. 3	20. 05	41. 0	0. 85	1. 7
2	20. 25	39. 2	28. 05	54. 4	3. 3	6. 4
3	24. 2	48. 6	24. 15	48. 5	1. 45	2. 9
4	18. 7	36. 5	29. 9	58. 3	2. 7	5. 2
5	29. 3	54. 3	22. 9	42. 4	1. 8	3. 3
6	20. 5	48. 6	20. 3	48. 1	1. 4	3. 3
7	24. 3	60. 5	14. 0	34. 8	1. 9	4. 7
8	24. 3	37. 1	39. 4	60. 2	1. 8	2. 7
9	17. 1	35. 7	28. 7	59. 9	2. 1	4. 4
10	17. 0	43. 6	20. 7	53. 1	1. 3	3. 3
Total	223. 7	461. 4	248. 15	500. 07	18. 6	37. 9
Mean	22. 37	46. 1	24. 815	50. 01	1. 86	3. 79

An examination of the data obtained by using the point-quadrat methods (table 3) discloses that, even when a minimum of 10 needles per half-square-meter quadrat is used, the percentage composition of the elements of the vegetation quite closely approaches that obtained from dry weights. So far as the four methods of employing the apparatus are concerned, the data are not sufficiently extensive, nor have they been sufficiently tested, to permit the drawing of inferences. Yet it is fairly clear that somewhat greater accuracy—assuming that the data obtained from dry weights provide a satisfactory basis for comparison—is obtained by the inclined method, where the needles are pushed to the ground and all hits tabulated (method B). Moreover, from these preliminary trials, it is also evident that the foregoing statement holds, though with some variation, for all the elements of the vegetation. No well-marked tendencies toward underhitting of legumes or overhitting of grasses are ascertainable. The addition of further readings from a second and third set within the half-square-meter area merely resulted in slight percentage changes, the net result of the total hits of 30 needles per quadrat being nearly the same as that from 10 needles. From these preliminary studies, and under the conditions described, it would appear that for sampling small areas a large number of readings is not necessary.

TABLE 3.—*Summary of data obtained by point-quadrat methods* [1]

Quadrat No.	Needles straight				Needles inclined 45°			
	A		B		A		B	
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition
	HITS ON LESPEDEZA							
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	4	40	21	45.7	[2] 4	40	[2] 17	40.5
2	5	50	[2] 25	59.5	[2] 6	60	[2] 13	44.8
3	2	22.2	6	18.8	4	40	12	33.3
4	4	40	22	61.1	[2] 4	44.4	[2] 21	51.2
5	4	44.4	15	57.7	6	60	24	70.6
6	5	50	13	38.2	2	20	12	35.3
7	0	0	5	21.7	[2] 2	22.2	[2] 22	48.9
8	[2] 4	44.4	[2] 12	63.2	[2] 1	10	[2] 15	40.5
9	[2] 2	20	[2] 8	25	[2] 4	40	[2] 12	26.7
10	5	55.6	14	46.7	[2] 4	44.4	[2] 17	45.9
Total	35		141		37		165	
Mean of 10 quadrats	3.5	36.7	14.1	43.7	3.7	38.1	16.5	43.8
Mean of 20 quadrats	3.8	41.0	13.8	46.5	3.6	37.0	15.4	48.0
Mean of 30 quadrats	3.9	39.1	14.4	45.7	3.6	36.4	15.5	41.3
	HITS ON GRASSES							
1	6	60	25	54.4	[2] 5	50	[2] 23	54.8
2	5	50	[2] 15	35.7	[2] 3	30	[2] 15	51.7
3	7	77.8	26	81.3	6	60	24	66.6
4	6	60	14	38.9	[2] 4	44.4	[2] 19	46.3
5	5	55.6	11	42.3	4	40	10	29.4
6	5	50	21	61.8	8	80	22	64.7
7	9	100	18	78.3	[2] 5	55.6	[2] 18	40
8	[2] 4	44.4	[2] 6	31.6	[2] 8	80	[2] 20	54.1
9	[2] 7	70	[2] 23	71.9	[2] 6	60	[2] 32	71.1
10	4	44.4	16	53.3	[2] 4	44.4	[2] 18	48.6
Total	58		175		53		201	
Mean of 10 quadrats	5.8	61.2	17.5	54.9	5.3	54.4	20.1	52.7
Mean of 20 quadrats	5.2	57.4	15.2	52.1	5.5	57.3	19.1	53.5
Mean of 30 quadrats	5.3	57.0	16.6	53.3	5.6	57.8	20.5	55.0
	HITS ON ALL FORBS							
Mean of 10 quadrats	0.2	2.1	0.4	1.3	0.7	7.2	1.4	3.7
Mean of 20 quadrats	.1		.4		.4		1.1	
Mean of 30 quadrats	.1		.3		.6		1.4	

---

[1] In this and subsequent tables A is used to indicate only the first hit on vegetation tabulated; B to indicate all hits on vegetation tabulated, as needle is pushed to the ground, irrespective of whether a given plant is hit once or oftener.

[2] One or more hits on forbs were obtained in this quadrat. Although not listed separately in a table because of their relative unimportance to the study as a whole, the hits on forbs were included in calculating percentage composition of lespedeza and grasses. These small percentages of forbs therefore account for the apparent discrepancies in the composition figures for lespedeza and grasses.

COMPARISON OF DIFFERENT METHODS OF USING THE POINT-QUADRAT APPARATUS FOR BOTANICAL ANALYSIS

PROCEDURE

In larger areas of the same pasture that was described in the first section of this report, a more extensive investigation was directed at comparing the 4 different applications of the point-quadrat method to which reference has already been made. Ten sample areas had been selected previously and covered with wire cages (approximately 4×4 feet) to exclude grazing animals. During early July, and again in August, the point-quadrat apparatus was set up at random in 2 separate places within the sample area. Readings were first taken with the needles inclined at an angle of 45°, and 2 sets of data, as before, were recorded. Without moving the front supports, the needles were then set vertically, and a second set of data was obtained. Following the completion of such readings, a rectangular area (6×19 inches), equivalent to that covered by vegetation through which the inclined needles passed, was carefully clipped, and the species later separated by hand. This procedure was repeated for the other set on the same plot, and then for each of the remaining 9 similar plots. A total of 200 needles for each of the 4 methods was thus obtained.

In August after the vegetation had had a month of undisturbed growth, the foregoing procedure was repeated for each sample area, except that the parts previously harvested were avoided in making the tests. By August the vegetation had attained a fairly uniform height of 6 inches, whereas in July the general level had been 4 inches. Since the vegetation was 2 inches taller in August, a slightly larger rectangular area (8 × 19 inches) was harvested for dry-weight determinations. By using such small harvested areas it was hoped that the correlation of percentage composition between the oven-dry weight, for a given element of the vegetation, and that determined by point-quadrat methods would be more precise, a relationship well demonstrated by the work of Arny and Schmid.[9] Furthermore, if greater precision were thus attained, the relative merits of the four methods tested would be thrown into sharper contrast and be of proportionately greater significance.

RESULTS

The data given in tables 4 and 5 summarize for lespedeza the results for July and August obtained by using the 4 techniques available with the point-quadrat method. Number of hits and percentage composition per quadrat (set of 10 needles) for lespedeza are here indicated. While the mean percentage composition contributed by lespedeza in July varies considerably with the method used, the standard deviation and standard errors clearly indicate that a more reliable result is obtained by recording all hits (method B), irrespective of whether the needles are vertical or inclined at an angle of 45°. Moreover, by comparing with these results the figures for July on percentage composition of lespedeza on a dry-weight basis (table 4) the greater reliability of this method (B) of counting all hits is further substantiated. The data for July appear to indicate that somewhat more satisfactory results can be obtained with the inclined method than with the vertical one, but the August data fail to confirm this finding. It should be recalled, however, that the vegetation in August was a good 2 inches taller, suggesting the possibility that in a vertical position the taller, flat-bladed lespedeza might have been hit more often than if the needles had been inclined.

---

[9] See footnote 6.

TABLE 4.—*Summary of data obtained in point-quadrat determinations for lespedeza in July*

Quadrat No.	Needles straight				Needles inclined 45°				Lespedeza composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Precent*
1	[1] 3	33.3	[1] 11	31.4	4	40	[1] 18	51.4	8.2	46.1
2	[1] 6	60	[1] 22	62.8	4	40	[1] 27	56.2	11.5	63.8
3	[1] 3	30	[1] 21	52	4	40	[1] 27	60	5.3	43.3
4	5	50	16	57.1	5	50	20	57.1	6.6	59.9
5	5	50	15	46.9	4	40	27	56.2	6.8	51.9
6	[1] 5	55.6	[1] 13	44.8	[1] 6	54.5	[1] 24	55.8	6.5	55.56
7	7	70	[1] 25	73.5	7	70	22	53.6	7.5	63.8
8	5	50	22	61.1	7	70	22	59.4	9.3	76.3
9	6	60	23	69.7	8	80	33	70.2	7.4	72.3
10	6	60	27	65.8	6	60	21	55.2	6.9	62.8
11	7	77.8	15	55.6	8	80	33	78.6	7.0	71.6
12	6	60	29	69.05	8	80	33	73.3	9.4	82.3
13	[1] 5	50	[1] 21	60.0	[1] 5	50	[1] 20	54	6.8	56.7
14	8	80	23	62.2	8	80	30	71.4	9.0	73.6
15	6	60	18	54.5	4	40	35	72.9	4.0	68.4
16	7	70	[1] 36	85.7	[1] 7	70	[1] 38	80.8	9.3	71.7
17	[1] 3	30	[1] 18	47.4	[1] 4	40	[1] 21	48.8	7.7	60.6
18	3	30	21	47.7	[1] 4	40	[1] 34	68	9.8	64.6
19	6	60	27	69.2	5	50	28	70	11.8	75.4
20	7	70	[1] 24	60	[1] 3	36	[1] 22	50	5.0	51.9
Total	109	--------	427	--------	111	--------	535	--------	158.6	--------
Mean	5.5	58.6	21.4	58.9	5.6	55.5	26.8	62.1	7.9	64.1
Standard deviation	--------	15.24	--------	12.19	--------	16.55	--------	9.96	--------	9.77
Standard error	--------	3.41	--------	2.70	--------	3.70	--------	2.23	--------	2.18

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 3.

The data for the grasses are given in tables 6 and 7. As in the case of lespedeza, these results indicate that for the grasses involved the counting of the total number of hits (method B) is more accurate than merely listing the first species hit per needle (method A). Standard deviations and standard errors both bear out this assertion. Moreover, the inclined method, under the conditions prevailing in July appeared to give slightly better estimates for the percentage composition furnished by grasses, as judged from the data for dry weight of grasses, given in table 6. The figures for August parallel those for lespedeza in showing that the most reliable results were obtained by counting the total hits when the needles were in a vertical position, but the differences obtained by this method and that in which the needles are inclined were slight. Both methods, as compared with dry-weight percentage figures, gave reliable results.

TABLE 5.—*Summary of data obtained in point-quadrat determinations for lespedeza in August*

Quadrat No.	Needles straight				Needles inclined 45°				Lespedeza composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	4	40	15	40.5	[1] 2	20	[1] 14	31.8	5.6	34.8
2	2	20	29	49.2	2	20	22	37.9	6.0	38.6
3	3	30	32	61.5	5	50	34	52.3	8.6	50.4
4	6	60	32	53.3	2	20	22	35.5	8.8	47.8
5	4	40	38	64.4	5	50	43	55.1	15.4	67.5
6	6	60	38	58.5	6	60	[1] 44	61.1	12.4	59.9
7	7	70	55	76.4	8	80	45	73.8	15.9	69.7
8	6	60	49	70	3	30	37	62.7	12.5	62.2
9	4	40	32	64	4	40	48	68.6	14.3	67.8
10	6	60	28	73.1	6	60	46	61.3	12.5	63.4
11	8	80	48	81.4	7	70	56	81.2	18.2	84.2
12	7	70	39	65	1	10	45	57.7	11.2	54.3
13	5	50	31	58.5	6	60	40	72.7	11.7	64.8
14	4	40	30	50	3	30	31	50	9.7	47.8
15	4	40	39	63.9	3	30	33	60	11.0	56.4
16	6	60	37	67.3	2	20	38	52.0	14.6	58.6
17	5	50	43	68.3	5	50	40	72.7	11.5	65.3
18	5	50	60	77.9	7	70	60	73.2	14.7	68.1
19	5	50	47	70.1	3	30	38	57.6	11.4	57.9
20	4	40	45	70.3	5	50	51	73.9	10.5	61.4
Total	101		777		85		787		236.5	
Mean	5.1	50.5	35.9	64.2	4.3	42.5	39.4	60.2	11.8	59.0
Standard deviation		14.68		10.32		20.23		13.64		11.35
Standard error		3.28		2.31		4.52		3.05		2.53

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 3.

TABLE 6.—*Summary of data obtained in point-quadrat determinations for grasses in July*

Quadrat No.	Needles, straight				Needles, inclined 45°				Grass composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	[1] 5	55.5	[1] 23	65.7	6	60	[1] 15	42.8	8.7	48.8
2	[1] 3	30	[1] 12	34.2	6	60	[1] 20	41.7	6.5	36.0
3	[1] 5	50	[1] 17	42	6	60	[1] 16	35.5	4.4	44.2
4	5	50	12	42.8	5	50	15	42.8	4.4	39.9
5	5	50	17	53.1	6	60	21	43.8	6.2	47.3
6	[1] 2	22.2	[1] 13	44.8	[1] 4	36.4	[1] 18	41.8	4.9	41.9
7	3	30	[1] 8	23.5	3	30	19	46.3	4.1	34.9
8	5	50	14	38.9	3	30	15	40.6	2.8	23.0
9	4	40	10	30.3	2	20	14	29.8	2.8	27.3
10	4	40	14	34.1	4	40	17	34.7	3.9	36.1
11	2	22.2	12	44.4	2	20	9	21.4	2.7	27.6
12	4	40	13	30.95	2	20	12	26.7	2.0	17.5
13	[1] 3	30	[1] 7	20.0	[1] 2	20	[1] 7	18.9	2.1	17.5
14	2	20	14	37.8	2	20	12	28.6	3.1	25.3
15	4	40	15	45.5	6	60	13	27.1	3.1	30.3
16	3	30	[1] 5	11.9	[1] 2	20	[1] 7	14.9	3.2	24.7
17	[1] 6	60	[1] 17	44.7	[1] 5	50	[1] 21	48.8	4.9	38.6
18	7	70	23	52.3	[1] 5	50	[1] 15	30	5.2	34.2
19	4	40	12	30.8	5	50	12	30	3.8	24.3
20	3	30	[1] 15	37.5	[1] 6	60	[1] 21	47.7	4.6	47.8
Total	79		273		82		299		83.4	
Mean	3.95	40.0	13.7	38.3	4.1	40.3	14.95	34.7	4.2	38.4
Standard deviation		13.52		12.21		16.86		9.99		9.82
Standard error		3.02		2.73		3.77		2.23		2.19

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 8.

TABLE 7.—*Summary of data obtained in point-quadrat determinations for grasses in August*

Quadrat No.	Needles, straight				Needles, inclined 45°				Grass composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	6	60	22	59.5	[1] 7	70	[1] 27	61.4	10.3	64.0
2	8	80	30	50.8	8	80	36	62.1	9.5	61.1
3	7	70	20	38.5	5	50	31	47.7	8.4	49.2
4	4	40	28	46.7	8	80	40	64.5	9.6	52.1
5	6	60	21	35.6	5	50	35	44.9	6.6	28.9
6	4	40	27	41.5	4	40	[1] 27	37.5	7.0	33.8
7	3	30	17	23.6	2	20	16	26.2	6.9	30.2
8	4	40	21	30	7	70	22	37.3	7.6	37.8
9	6	60	18	36	6	60	22	31.4	6.8	32.2
10	4	40	14	26.9	4	40	29	38.7	7.2	36.5
11	2	20	11	18.6	3	30	13	18.8	3.4	15.8
12	3	30	21	35	9	90	33	42.3	9.4	45.6
13	5	50	22	41.5	4	40	15	27.3	6.2	34.3
14	6	60	30	50	7	70	31	50	10.5	51.8
15	6	60	22	36.1	7	70	22	40	8.5	43.6
16	4	40	18	32.7	8	80	35	48.0	10.3	41.4
17	5	50	20	31.7	5	50	15	27.3	6.1	34.7
18	5	50	17	22.1	3	30	22	26.8	6.9	31.9
19	5	50	20	29.9	7	70	28	42.4	8.3	42.1
20	6	60	19	29.7	5	50	18	26.1	6.6	38.6
Total	95		418		114		517		156.1	
Mean	4.8	50.0	20.9	35.8	5.7	57.0	25.9	40.0	7.8	40.3
Standard deviation		14.68		10.32		19.76		13.03		11.45
Standard error		3.28		2.31		4.42		2.91		2.56

[1] 1 or more hits on forbs were obtained in this quadrat. See footnotes of table 3.

From the data given in tables 4, 5, 6, and 7, it is clear that, at least for the heights of vegetation considered, there is no necessity for the computation of correction factors. These might be required, however, for a finer-leaved grass element.

Although some forbs were sampled and their percentage composition calculated as for lespedeza and the grasses, the mean percentage, as determined on a dry-weight basis, was 2.4 for July and 0.67 for August. Therefore it does not seem necessary to consider them in detail. In general, it might be remarked that the percentage composition of the vegetation furnished by forbs was more accurately sampled by the inclined-point method wherein all hits were tabulated.

A consideration of the factors of time and ease of operation of the various methods was outside the immediate objective of this study. Yet, as shown by other investigators, including Tinney, Aamodt, and Ahlgren,[10] the use of the point-quadrat method, regardless of what special application may be made of the apparatus for a particular investigation, is definitely time- and labor-saving as compared with most other methods of botanical analysis. So far as counting all hits versus counting only the first plant hit per needle is concerned, the latter method is undoubtedly the more rapid; but, as indicated by the general results of this study, greater accuracy is obtained by counting all hits per needle as the latter is pushed into the soil. The particular method to be employed will depend to a considerable extent on the degree of accuracy required in a given investigation.

---

[10] See footnote 4.

SUMMARY

During July and August 1942 a study of the uses of the point-quadrat method of botanical analysis of a lespedeza-grass pasture mixture was undertaken (1) to determine the relative merits of this general method as compared with those of the count-list method, and (2) to ascertain the relative efficiency of four different applications of the point-quadrat method, for the same vegetation.

In a study made on one-half-square-meter quadrats, the results, though not subjected to statistical analysis, indicate that, for the vegetation analyzed, all four applications of the point-quadrat method yield more satisfactory results than the count-list method, assuming that weight-list data furnish a satisfactory basis of comparison. Moreover, no tendencies toward underhitting of the legume or overhitting of the grass were indicated in the results so that evaluation of correction factors do not appear necessary.

Results obtained from studies carried out in July and August, suggest that on the low-growing vegetation of the tests, the counting of all hits as the needles of the point-quadrat apparatus are pushed through the plants to the ground yields more reliable results than merely recording the first plant hit by each of the 10 needles. For the lower-growing (about 4 inches tall) vegetation in July, the inclined-point method gave more satisfactory results, as judged from calculated standard errors, than the straight method. Yet, in August, with the vegetation some 2 inches taller, the reverse appeared to be true. Height of the vegetation, as well as the morphology of the species involved, thus appeared to influence the manner of the use and the evaluation of results obtained by means of the point-quadrat method. Thus, it is likely that the most satisfactory method of botanical analysis will vary with the type of vegetation, and that this method should be evaluated on the basis of repeated trials.

REGRESSION OF INSECT DENSITY ON DISTANCE FROM CENTER OF DISPERSION AS SHOWN BY A STUDY OF THE SMALLER EUROPEAN ELM BARK BEETLE[1]

By F. M. WADLEY, *statistical consultant*, and D. O. WOLFENBARGER,[2] formerly *senior field assistant, Division of Japanese Beetle Control, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In a study of the smaller European elm bark beetle (*Scolytus multistriatus* (Marsham)) in its role as a vector of the Dutch elm disease, the writers have secured data showing distribution patterns around centers of dispersion. The results are of practical importance from the standpoint of control in indicating distance of spread and any directional tendencies that may appear. They also give an idea of the form of regression of population density on distance, a subject that is of considerable interest in insect ecology. Critical statistical methods are needed for such a study. Records of insect dispersion occur frequently in entomological literature, but usually without sufficient quantitative information for analysis. "Dispersion" as the term is here used includes active or passive movement resulting in distribution and has a broader meaning than that given to "migration" by Williams (*13*).[3] The subject has recently been reviewed by Whitfield (*12*).

In dispersion radiating from a center several elements will contribute to the observed change in insect density with distance. In the first place density will grow less as the circumference expands. This by itself should give a density having a positive linear relation to the inverse of distance, and its relation to distance itself would be of hyperbolic form. Secondly, the trend will be modified by various factors, chiefly the tendency of insects to stop on finding satisfactory places. This tendency will cut down the absolute numbers, probably rapidly at first and more slowly later. Finally, the density will often fall, not to zero, but to some low level characteristic of the general region. Centers of positive attraction, or a marked directional reaction, may obscure this picture, but in many cases it will probably hold. Observation of some effect of the insects' activities may serve almost as well as direct counts to evaluate density.

In studying records in the literature on various insects, as well as the bark beetle data, the writers decided not to attempt at first to fit a curve adapted to the three elements named. The second element seemed too uncertain in its action and in its relation to the first. Precise data in sufficient quantity were lacking. Distance must evidently have a twofold effect, but the inverse of the square of the distance did not show a usable relation. An empirical study of trend seemed best at first.

[1] Received for publication May 22, 1943.

[2] The writer is now assistant entomologist with the Division of Domestic Plant Quarantines.

[3] Numbers in parentheses refer to Literature Cited, p. 307.

Journal of Agricultural Research, Washington, D. C.
Vol. 69, No. 7 Oct. 1, 1944 Key No. K-333

Preliminary plotting suggested curves of gently declining slope. Later the simple semilogarithmic curves were used, and more complex curves were tried. In most cases the form of the data determines at once which of the equations $Y=a+b \log X$ or $\log Y=a+bX$, where X is distance and Y is density, should be used. Curves of the type used in this study can apparently be fitted fairly well by either (*4*). Frampton, Linn, and Hansing (*5*), dealing with a plant-disease spread by insects, used logarithms of density and related this curve form to that of diffusion. Their article contains a detailed mathematical development. Their case differs somewhat from the one studied by the writers in that it deals with infiltration from a large area rather than dispersion from a small center.

When logarithms of distance are used, no reasonable interpretation can be made at zero distance, and the curve reaches zero density at some finite distance. When logarithms of density are used, no such zero density is reached. These are trivial objections in a preliminary study of trends.

TENDENCIES SHOWN BY SOME PREVIOUS DISPERSION RECORDS

In dispersion data on codling moth adults given by Van Leeuwen (*11*), density showed a fairly close linear relationship to the logarithm of distance. Steiner (*10*) showed a somewhat similar relationship in the dispersion of the newly hatched larvae. Gaines (*6*) studied boll weevil activity as related to distance from hibernation quarters, McColloch (*8*) studied dispersion of the hessian fly, and Eckert (*3*) studied flight distances of bees. Other data on dispersion are given by Hawley (*7*) on Japanese beetle larvae, and by Wolfenbarger (*14*) on potato flea beetles as measured by larval injury. Annand, Chamberlin, Henderson, and Waters (*1*) showed the decrease in incidence of curly top in beans as an index of beet leafhopper abundance.

The graphs of all these records showed similar trends. There was a steep falling off from the center, or point of origin. As the distance increased, the slope became less steep although tending toward a low level. When the data were plotted on semilogarithmic paper, the trend approximated a straight line. The logarithmic plotting, however, did not always give a straight line all the way, as the curve often flattened out when a low level was reached. This flattening may perhaps be related to the general population level characteristic of the region. Often only a few points were available from which to plot the curve; therefore, the data were frequently not suitable for intensive study. In some of these cases the conditions were similar to those studied by Frampton, Linn, and Hansing; some were typically dispersions from a center.

AREAS UNDER OBSERVATION

In the authors' work on *Scolytus multistriatus* exceptional opportunities to observe dispersion from a center were found in two locations. In both places considerable emergence from a known center occurred. The index of activity used was the feeding in twig crotches by newly emerged adults. Only injuries that could obviously have been made by adults from the observed source were counted.

Crotch feeding has been observed to be a better criterion of general dispersion than the formation of egg galleries. The latter activity is strongly influenced by the attractiveness of suitable trees or parts of trees, but crotch feeding by new adults seems to occur rather universally in elms. The uneven distribution of elms in the terrain caused some irregularity in the data, as isolated elms standing in open spaces were attacked more than others. However, the most important features of dispersion were sufficiently marked to appear even with this irregularity.

The first location was in a wide valley in northern New Jersey where abundant elm slash had been left in a small area. *Scolytus multistriatus* had bred in enormous numbers, and twig injury was unusually abundant. The country was rather open, with scattered trees, elms being fairly numerous. Examination of 70 trees 5 inches or more in diameter was made at definite distances in 5 directions from the center to 1,200 feet; 1 tree was examined at 1,300 feet and 3 more at 2,600. Distances were measured by pacing from the edge of the small area of heavy breeding. The trees were sampled by examining branches taken from the upper part of the crown. On each branch taken, all twig-crotch injuries of the year of exceptional beetle flight were counted. Usually 100 or more crotches were examined per tree. The type of injury and the method of examination and determination of year of injury have been described by Wolfenbarger and Buchanan (*15*). In this area the injury, although several years old when examined late in 1938, could be determined with certainty. The use of the percentage of crotches attacked in each tree as an index of activity compensates for the unequal size of the samples and seems the best measure of effect. Exclusion of small trees helps to make the data comparable.

The second location was in southwestern Connecticut. The terrain was hilly, with woodland to the west and farms with scattered trees to the east. The beetles had been active the previous year. Early in 1939 examinations were made on 108 trees 4 inches or more in diameter, in several directions, as in the New Jersey area. A number of trees were selected up to 3,000 feet from the center and some others from 5,000 to 7,200 feet. Several hundred crotches were examined in every tree, and as many as 2,000 in distant trees very lightly attacked. *Scolytus multistriatus* was not present in appreciable numbers except in the center of dispersion, as determined by intensive study of the location and general examination of the surrounding area.

RESULTS IN THE NEW JERSEY AREA

In the New Jersey area a comparatively rapid rate of decrease in the number of injured twig crotches was observed as the distance from the center increased. At the center an average of nearly 40 percent of the tree crotches were injured and all trees were heavily attacked. When a distance of over 600 feet had been reached, however, the average was only 2 to 4 percent, and little further decrease in the proportion of injured crotches was noted. There was considerable variation among individual trees. The highest percentage from a tree between 600 and 1,000 feet from the center was 8.7. At distances of 1,000 to 1,300 feet 15 trees were examined; in one, at 1,025 feet, the injury was 18 percent; in another, at 1,180, it was 12 percent; no other injury higher than 5 percent was recorded. These high individual values may have been due to favorable location or attractiveness or to secondary centers. The few trees at 2,600 feet showed about 3 percent attack. In table 1 are brought together averages from 70 trees, arranged by directions and distance groups. From 1 to 7 trees are represented by each entry in the table.

TABLE 1.—*Percentages of crotches per tree injured by Scolytus multistriatus, classified according to distance and direction from the source, New Jersey area*

Distance (feet)	Northwest	West	Southeast	Northeast	Southwest	Average [1]
0-50	36.4	35.8	29.9	33.8	36.4	35.5
51-200	18.7	14.0	30.2		9.6	17.3
201-400	9.4	6.1	20.3			12.4
401-600	11.0	2.8	16.2		1.0	10.4
601-800	1.2	2.5	2.2		2.3	2.1
801-1,000	1.6	1.3	4.3	0.9		2.7
1,001-1,200		2.0	2.1	8.1	2.2	3.8

[1] Averages weighted by the number of trees in each distance and direction class.

To test the significance of the dispersional differences between distances and between directions, the data were statistically examined by the method of analysis of variance (table 2). As many of the percentages were less than 10, they were converted into equivalent angles according to the method described by Bliss (*2*). A special treatment for uneven frequencies (*9*, *Sec. 11.9*) was adopted. The fact that not all directions are represented in all distance classes makes analysis difficult. The apparent importance of distance makes it necessary to allow for it in any analysis of direction. The percentages of injured crotches for four distance classes (0-50, 51-200, 401-600, and 601-800 feet) and four directions (northwest, west, southwest, and southeast) were used for analysis, because for these classes data were available for all combinations of direction and distance. In these data 35 trees were represented and the larger part of the important observations were included. The class at 201-400 feet, which was omitted, contained only 6 trees, and the northeast class ,0-50 feet, only 2; these appeared to show no tendencies differing much from those shown by the other classes and directions under observation. It was necessary to use a few classes with only 1 tree each, but this is not believed to have biased the error estimate.

TABLE 2.—*Analysis of variance of angles corresponding to percentages of crotches attacked, New Jersey area*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between directions	3	123.77	41.26
Between distances	3	1,779.17	593.06
Interaction	9	231.81	25.76
Error	19		19.57

Distance shows a strong, clear-cut influence, but there is little evidence of the influence of direction. According to table 1, the southeast direction showed, on an average, higher percentages of crotches attacked, but the individual tree injuries in this direction varied considerably. When distance classes including all trees out to 1,000 feet from the center were analyzed without regard to direction, highly significant differences were found. Beyond about 600 feet no significant difference among distance classes could be demonstrated.

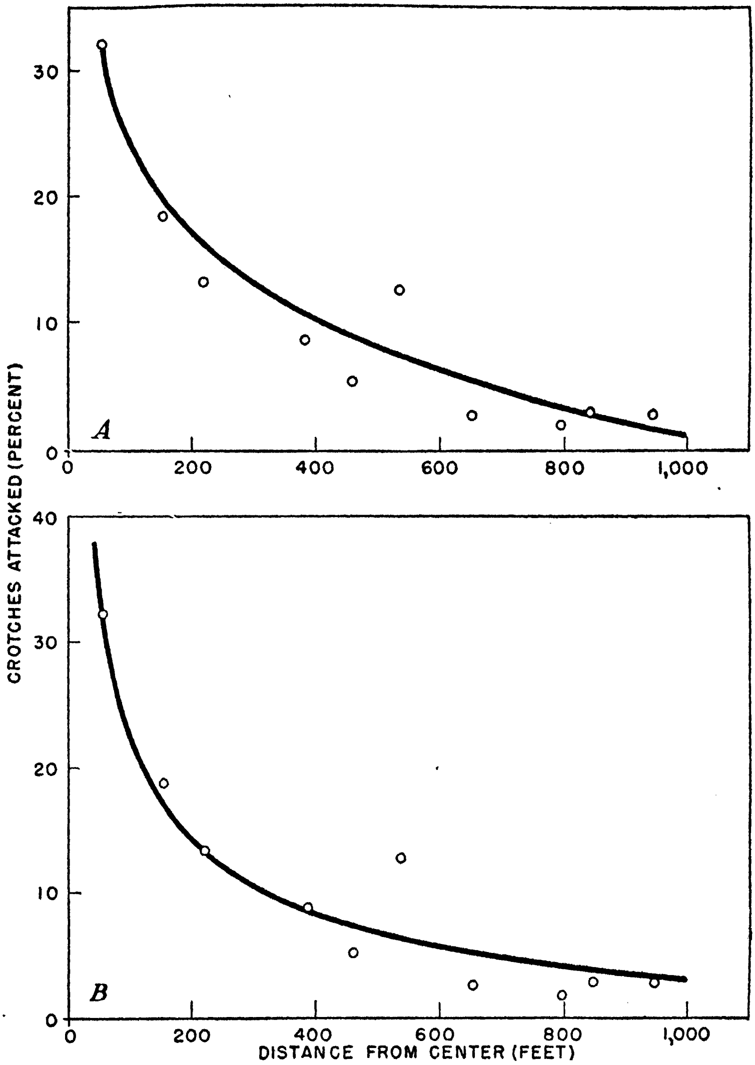


FIGURE 1.—Regression of percentage of crotches fed upon by *Scolytus multistriatus* on distance from breeding center, New Jersey area. *A*, Y values obtained from the equation $Y=71.56-23.55 \log X$, plotted against X; *B*, Y values from the equation $Y=27.94-8.71 \log X+1276/X$, plotted against X. The small circles are group means for trees 50–100 feet from the center and then at 100-foot intervals out to 1,000 feet.

It is true that distance classes could differ without showing a real regression. Preliminary plotting of individual percentages against distance indicated a definite slope, with a strong suggestion of curvilinearity, but much individual variation. The high percentages in several trees near the 600-foot mark (one reached 17 and one 33 percent) bring that class to a higher level than one or two classes closer to the center. Linear regression was investigated first, because its significance is easily determined and because it furnished a standard for testing curvilinearity.

The trees from 50 to 1,000 feet were used, 44 being available, without discrimination as to direction. Trees nearer than 50 feet were in the margin of the breeding center, while those beyond 1,000 feet seem to be pretty well away from the influence of the center and to express the probable regional level. Regression was calculated by least-square methods from individual tree values. The simple linear regression was $Y=23.85-0.0254X$, Y being the percentage of crotches fed on and X the number of feet from the center of dispersion. The standard error of estimate was 8.47 percent, the standard error of the regression coefficient about 0.004. Since the regression coefficient is over six times as large as its standard error, it may be regarded as highly significant in its difference from zero.

Next the curvilinearity was tested. The standard error of estimate in respect to the equation $\log Y=1.408-0.00127X$ was 8.27 percent, a value not essentially different from that obtained from the straight line. The equation $Y=71.56-23.55 \log X$ gave a standard error of estimate of 7.22 percent. Lastly, the equation $Y=27.94-8.71 \log X+1276/X$ gave a standard error of estimate of 6.76 percent.

Curves obtained by plotting the estimated Y values from the last two equations against X are illustrated in figure 1. The curves are somewhat similar, although the curvature is more pronounced in *B* and the observations up to 500 feet fit this curve better than the one in *A*.

The advantage gained by the adoption of each curve over the one preceding it may be seen in the successive reductions in the sum of the squared deviations. The sum of the squared deviations from the mean is 5,837. Linear regression reduces this value to 3,012, the equation represented in figure 1, *A*, gives a value of 2,189, and that represented in *B* gives 1,871.

It is concluded that (1) directional differences are not apparent, (2) as the distance from the source increases, the number of crotches fed upon decreases, (3) with few exceptions the regional level is reached at 601-800 feet from the center, and (4) the regression is curvilinear.

RESULTS IN THE CONNECTICUT AREA

In the Connecticut area feeding was less intense; trees at the very center had 15 to 20 percent of their crotches attacked, and averages fell rapidly as the distance increased. More crotches were examined per tree than in New Jersey. Of 26 trees between 400 and 1,000 feet from the center, 1 had 5.8 percent of the crotches attacked (890 feet), 1 had 1.6 percent (470 feet), and 1 had 1.2 (671 feet); all the others had less than 1 percent. Of 30 trees between 1,000 and 2,000 feet from the center, only 4 had 1 percent or more of the crotches attacked, the maximum being 3 percent. Over 2,000 feet from the center 20 trees were examined. None had as many as 1 percent of the crotches attacked, but about half showed some injury. The most distant tree (7,180 feet) showed 0.2 percent of the crotches attacked. If, as is believed, the beetles from the center were almost the only ones in the area, spread of over a mile by individuals is suggested.

Class averages of percentages from the 58 trees up to 1,000 feet are shown in table 3. Only three directions are shown; the southeast direction was examined also, but no trees occurred nearer than 1,000 feet.

TABLE 3.—*Percentages of crotches per tree injured by Scolutus multistriatus, classified according to distance and direction from the source, Connecticut area*

Distance (feet)	Northwest	Southwest	Northeast	Average[1]
0–50			10. 41	10. 41
51–100	4. 18	5. 30	6. 19	5. 44
101–200	2. 60	3. 18	1. 29	2. 40
201–400		1. 56		1. 56
401–600	. 20	1. 09		. 44
601–800	. 32	. 15		. 18
801–1, 000	1. 01			1. 01

[1] Averages weighted by the number of trees in each distance and direction class.

Only two distance classes, 51–100 feet and 101–200 feet, have all three directions represented; 17 trees are included in these 6 groups, and these were analyzed as before (table 4).

TABLE 4.—*Analysis of variance of angles corresponding to percentages of crotches attacked, Connecticut area*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between directions	2	10. 26	5. 13
Between distances	1	28. 56	28. 56
Interaction	2	7. 90	3. 95
Error	11		13. 55

As before, no directional influence is seen. The influence of distance in these two classes is not significant. A more extended analysis carried out without regard to direction showed the difference between distance classes to be highly significant. With 43 trees at distances up to 600 feet, the mean square between distances was 166 with 6 degrees of freedom, and the error mean square was 25 with 36 degrees. Beyond 400 feet no significant difference between distance classes was shown. Observations more than 1,000 feet from the center showed no tendencies differing from the ones already noted.

Regression equations were calculated as before. Preliminary plotting indicated that the regression of percentages of crotches fed upon per tree on distance from the center was similar to that obtained with the New Jersey material. The linear regression coefficient was -0.0124 ± 0.0029 percent. The reality of a negative regression is thus clearly shown. Observations from 42 trees within the range of 7 to 600 feet furnished the data for the analyses. The following equations, together with their standard errors of estimate, were obtained:

Equation	Standard error of estimate
$Y = 6.31 - 0.0124\, X$	3. 65 percent
$Y = 18.19 - 6.77 \log X$	2. 96 percent
$Y = 10.77 - 3.84 \log X + 69/X$	2. 74 percent

The curve obtained by plotting the estimated Y values from the last equation against X is illustrated in figure 2. Residual sums of squares are as follows: From mean, 733; from straight line, 533; from semilogarithmic curve, 349; from modified semi-logarithmic curve, 292. Again significant improvement is shown in each step.

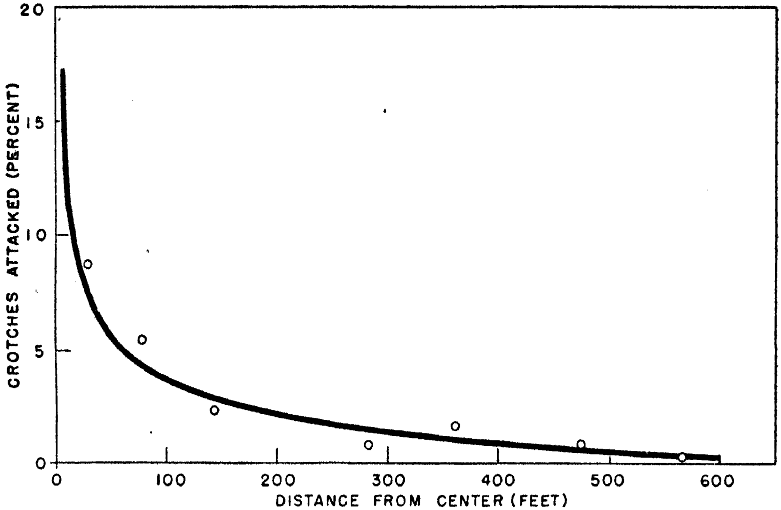


FIGURE 2.—Regression of percentage of crotches fed upon by *Scolytus multistriatus* on distance from breeding center, Connecticut area. Y values obtained from the equation $Y=10.77-3.84 \log X + 69/X$, plotted against X. The small circles are group means for trees at 7–50 and 51–100 feet from the center and then at 100-foot intervals to 600 feet.

Since it appeared that the beetles from the center under consideration were practically the only ones in the area, there was little question of a regional level. Hence curve fitting might have been extended farther from the center, probably with increased advantage for the curve over the straight line. However, such extension would lead to inclusion of a number of very low percentages and seemed unlikely to show any tendencies not already brought out.

DISCUSSION

Because of the consistency in the observations in New Jersey and Connecticut, we can regard the curvilinear relation between insect density and distance as established, and the curve of the equation $Y=a+b \log X+c/X$ as very promising for representing the relation. Several other curves allowing twice for effect of distance were tried with the New Jersey and Connecticut data, but none gave such good results as the modified semilogarithmic curve. This curve, however, cannot be regarded as precisely adapted to the factors affecting density. If allowance is made for increase in periphery, the numbers reaching various distances will form a frequency distribution that increases at first and then decreases.

SUMMARY

Following a consideration of the factors that may govern curves of insect dispersion, two pronounced infestations of *Scolytus multistriatus* (Marsham), one in New Jersey and the other in Connecticut, were studied, with twig feeding by adults as a measure of insect density. In neither infestation could indications of directional influence be found. In each case, however, pronounced falling off in density with increasing distance from the center was noted, the downward slope of the plotted curve tending to level off as the distance increased. At 401–800 feet from the center a low level of population was reached and farther out decline was hardly detectable, except for a few individual trees. The high level of general activity around the center extended only a few hundred feet. Indications were that individual beetles might travel more than a mile. In the range from the center to the zone where leveling off was marked, the decline showed in each location an undoubted curvilinear regression, although with variable material and moderate numbers its advantage over linear regression was not pronounced. Best results in fitting were given by the curve of the equation $Y=a+b \log X+c/X$, in which X is distance and Y the percentage of crotches attacked.

LITERATURE CITED

(1) Annand, P. N., Chamberlin, J. C., Henderson, C. F., and Waters, H. A.
1932. MOVEMENTS OF THE BEET LEAF HOPPER IN 1930 IN SOUTHERN IDAHO U. S. Dept. Agr. Cir. 244, 24 pp., illus.

(2) Bliss, C. I.
1938. THE TRANSFORMATION OF PERCENTAGES FOR USE IN THE ANALYSIS OF VARIANCE. Ohio Jour. Sci. 38: 9–12, illus.

(3) Eckert, J. E.
1933. THE FLIGHT RANGE OF THE HONEYBEE. Jour. Agr. Res. 47: 257–285, illus.

(4) Ezekiel, M.
1930. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London.

(5) Frampton, V. L., Linn, M. B., and Hansing, E. D.
1942. THE SPREAD OF VIRUS DISEASES OF THE YELLOWS TYPE UNDER FIELD CONDITIONS. Phytopathology 32: 799–808, illus.

(6) Gaines, J. C.
1932. STUDIES ON THE PROGRESS OF BOLL WEEVIL INFESTATION AT VARIOUS DISTANCES FROM HIBERNATION QUARTERS. Jour. Econ. Ent. 25: 1181–1187, illus.

(7) Hawley, I. M.
1934. A PRELIMINARY REPORT ON THE HORIZONTAL MOVEMENT OF GRUBS OF THE JAPANESE BEETLE. Jour. Econ. Ent. 27: 503–505.

(8) McColloch, J. W.
1917. WIND AS A FACTOR IN THE DISPERSION OF THE HESSIAN FLY. Jour. Econ. Ent. 10: 162–168, illus.

(9) Snedecor, G. W.
1940. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. Ames, Iowa.

(10) Steiner, L. F.
1939. DISTANCES TRAVELED BY NEWLY HATCHED CODLING MOTH LARVAE. (Scientific note) Jour. Econ. Ent. 32: 470.

(11) Van Leeuwen, E. R.
1940. THE ACTIVITY OF ADULT CODLING MOTHS AS INDICATED BY CAPTURES OF MARKED MOTHS. Jour. Econ. Ent. 33: 162–166.

(12) Whitfield, F. G. S.
1939. AIR TRANSPORT, INSECTS AND DISEASE. Bul. Ent. Res. 30: 365–442, illus.

(13) Williams, C. B.
1923–24. RECORDS AND PROBLEMS OF INSECT MIGRATION. Roy. Ent. Soc., London, Trans. 1923: 207–233, illus.

(14) Wolfenbarger, D. O.
1940. RELATIVE PREVALENCE OF POTATO FLEA BEETLE INJURIES IN FIELDS ADJOINING UNCULTIVATED AREAS. Ent. Soc. Amer. Ann. 33: 391–394, illus.

(15) ——— and Buchanan, W. D.
1939. NOTES ON ELM TWIG CROTCH INJURIES PRODUCED BY SCOLYTUS MULTISTRIATUS MARSHAM. Jour. Econ. Ent. 32: 377–381, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., OCTOBER 15, 1944 NO. 8

THE RING ROT BACTERIUM IN RELATION TO TOMATO AND EGGPLANT[1]

By R. H. LARSON

Instructor, Department of Plant Pathology, Wisconsin Agricultural Experiment Station

INTRODUCTION

Bacterial ring rot, incited by *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. and Burkh. is the most serious of recently introduced diseases of the potato (*Solanum tuberosum* L.). It has been reported as occurring in nature only on the potato. Spieckermann and Kotthoff [2] reported negative results in artificial inoculations of aboveground stems of potted plants of tomato (*Lycopersicon esculentum* Mill.) and related species, *L. humboldtii* (Willd.) Dunal, and *L. pimpinellifolium* Mill. (*L. racemigerum* Lange) in the field during 1910 and 1911. However, in 1913, in additional inoculations to 19 field-potted plants each of *L. esculentum* and *L. racemigerum*, 9 "diseased plants" in each of the 2 lots were recorded. No mention was made, however, of plant symptoms or of reisolation of the organism. *Solanum commersonii* Dunal and *S. citrullifolium* A. Br. were also reported as "susceptible."

Using tomato as a test plant, Stapp [3] observed occasional local withering of the leaves of young transplants a short distance above and below the point of stem inoculation. Reisolations were made after 3 to 4 months, at distances of 20 and 25 cm. below the site of the inoculation. Fruit infection was not reported and the organism was regarded as being "mildly pathogenic." In inoculation tests on field pea (*Pisum arvense* L.) and bean (*Phaseolus vulgaris* L.), the ring rot organism proved to be "slightly pathogenic." *Corynebacteruim sepedonicum* was reisolated from the three hosts infected. More recently Savile and Racicot [4] reported that the pathogenicity of the bacterial ring rot organism in young artificially inoculated tomato plants was markedly greater than that described by Stapp. They pointed out that symptoms of leaf wilting were evident in 19 days, at which time the tissues were quite heavily invaded by the bacteria. All tomato plants inoculated were severely affected, extensive yellow-walled stem cavities being formed, after which the plants soon died.

During early studies on the host range of the ring rot organism in the greenhouse at Madison, Wis., it was found that young tomato transplants (variety, Bonny Best) were very easily infected. Artifi-

---

[1] Received for publication May 21, 1943. The photographs that accompany this paper were made by Eugene H. Herrling.

[2] SPIECKERMANN, A., and KOTTHOFF, P. UNTERSUCHUNGEN ÜBER DIE KARTOFFELPFLANZE UND IHRE KRANKHEITN. I. DIE BAKTERIENRINGFÄULE DER KARTOFFELPFLANZE. Landw. Jahrb. 46: [659]-732 illus. 1914.

[3] STAPP, C. BEITRÄGE ZUR KENNTNIS DES BACTERIUM SEPEDONICUM SPIECKERM. ET KOTTH., DES ERREGERS DER "BAKTERIENRINGFÄULE" DER KARTOFFEL. Ztschr. f. Parasitenk. 2: [756]-823, illus. 1930.

[4] SAVILE, D. B. O., and RACICOT, H. N. BACTERIAL WILT AND ROT OF POTATOES. Sci. Agr. 17: 518-522, illus. 1937.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 8
Oct. 15, 1944
Key No. Wis.-147

cially inoculated test plants on being held in a warm greenhouse (24° C.) for a short period developed conspicuous wilting and withering, followed by unilateral chlorotic bleaching of the upper leaflets. A preliminary report[5] on bacterial ring rot in relation to the tomato has been published. The present paper is a report of investigations on the relation of the ring rot organism to the host tissues of tomato, on the disease development and resistance in tomato and eggplant, and on host range studies.

MATERIALS AND METHODS

Tomato plants (variety Bonny Best) used in the histological phase of this study were grown both in the greenhouse and in the field. The culture of the organism was a single-colony isolate from a fourth serial dilution plate of a laboratory stock culture isolated originally from an affected tuber collected in north-central Wisconsin and held in storage at 5° C. in inoculated Irish Cobbler potato tubers. The inoculum used in all cases was from 7-day-old pure culture on freshly prepared slants of potato dextrose agar containing neutral yeast extract grown in the dark at 20° to 22° C. Inoculations were made either by dipping a needle point into the culture and inserting it directly into the vascular region or by puncturing with a needle through a water suspension of the organism into the vascular tissue of the lower stem. Both types of inoculation were very successful. All greenhouse-inoculated test plants were held at an air temperature of about 24° C.

The plant tissues selected for histological study were immediately killed and fixed by placing sections directly in a solution consisting of 10 cc. of commercial 40-percent formalin, 5 cc. of glacial acetic acid, and 100 cc. of 50-percent ethyl alcohol. All tissues were evacuated under light suction while in the fixative. The fixed material was dehydrated following the tertiary-butyl-alcohol schedule and embedded in tissue-mat paraffin with a melting point at 52° to 54° C. according to the usual procedure. Transections and longisections were cut 8 to 10 microns in thickness and mounted serially to facilitate more complete observations on the path of vascular invasion by the bacteria. Miscroscopical examinations of histological material were in most cases paralleled by isolations from similar portions of the host tissues.

Reed's[6] Gram-stain formula and schedule were followed for the bacteria, which are strongly Gram-positive. The host tissues were then differentiated by counterstaining with safranine (1 percent in 95-percent ethyl alcohol) followed by fast green (saturated solution in absolute ethyl alcohol). This combination was found to be most satisfactory. In sections stained by this method the vessel walls retained a brilliant red, while the walls of the parenchymatous cells were differentiated by a deep green. This counterstaining in no way modified the preliminary deep-blue Gram stain of the bacteria in the host tissues.

---

[5] Larson, R. H., Walker, J. C., and Fogelberg, S. O. BACTERIAL RING ROT IN RELATION TO THE TOMATO. (Abstract) Phytopathology 31: 14–15. 1941.

[6] See Racicot, H. N., Savile, D. B. O., and Conners, I. L. BACTERIAL WILT AND ROT OF POTATOES—SOME SUGGESTIONS FOR ITS DETECTION, VERIFICATION, AND CONTROL. Amer. Potato Jour. 15: 213–318, illus. 1938.

EXPERIMENTAL RESULTS

INOCULATION AND ISOLATION

The bacterial organism was consistently recovered from the vascular system of stem and petiole of tomato plants artificially inoculated with the organisms. Transfers from resulting isolations were checked by needle inoculations to stems of known healthy potato test plants (varieties Green Mountain, Katahdin, and Triumph) on which typical systemic vine and tuber symptoms of bacterial ring rot were readily produced. In all cases the isolates from infected tomato plants exhibited in the potato the same degree of virulence as did the original culture.

SYMPTOMS

Symptoms in tomato plants systemically infected with the bacterial ring rot organism were of two distinct phases consisting of an early wilting and withering stage and a necrotic to moribund stage. The earliest symptoms of systemic invasion were evident within 8 to 12 days after inoculation, as a dull or dark-green coloration and slight flaccidity of the young apical leaflets. Concurrently a gradual irregular wilting, loss of firmness and drooping of the lower leaves was

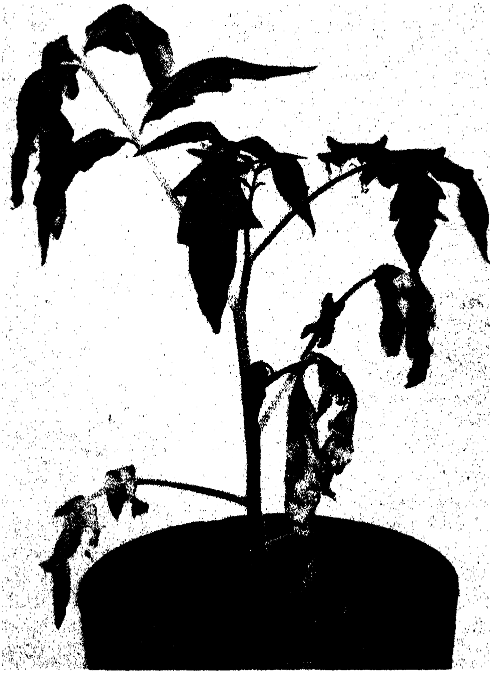


FIGURE 1.—Systemic infection of young Bonny Best tomato plant, growing in the greenhouse at 24° C., 4 weeks after inoculation of the lower stem with the bacterial ring rot organism. Irregular drooping and wilting of the upper leaves, necrosis of lower leaves, and slight petiole epinasty are evident.

evident, accompanied by a slight upward and inward marginal rolling of the lower leaves and subsequent slight downward turning of the petioles (fig. 1). Infected young tomato transplants, however, did not show a marked stimulation response of petiole epinasty preceding or following leaf wilting or flaccidity. On subsequent days as systemic invasion progressed irregular mottling approaching chlorosis appeared in the lower leaves as the color of the entire plant partly or wholly changed to a pale green with bleaching of the interveinal areas. Infected plants were more or less stunted. Infection commonly progressed on one side of the stem to adjacent petioles, rachis, and petiolule, causing a conspicuous retarded leaflet expansion and unilateral leaf development (fig. 2, *A*). Following these symptoms, the invaded plants took on a yellow cast with marginal leaf rolling and interveinal bleaching or browning. In addition, slight irregular necrotic streaking commonly occurred on the stems. This type of stem streaking was often followed by a conspicuous exudation of creamy-white or yellowish, slimy bacterial masses at the points of tissue break-down in the nodal region where the bacteria had spread outward through the cortical stem tissue (fig. 2, *B*). There was not a strong tendency, however, for the bacteria to come to the surface through fissuers in the stem or petiole tissues. When stems of infected plants were cut lengthwise, slight disintegration of the pitch cylinder in the form of small creamy-white to yellowish cavities were visible. Transections of the petiole and rachis, on the other hand, showed only a very slight water-soaked area adjacent to the vascular elements, in which could be seen a creamy-white bacterial ooze when slight pressure was applied.

Older plants showed noticeable stunting with shortened and somewhat thickened internodes. In cases of severe systemic infection an irregular wilting occurred in the upper portion of the plants when the only sign of infection was a slight chlorotic, shriveled condition of the lower leaves. Infected plants in the field which did not succumb early in the season showed the most pronounced symptoms (fig. 3). Pedicel and flower drop in infected plants in the field were not very common.

Distortion of infected fruit was not observed. However, internal fruit infection was manifest by the outward appearance of somewhat shriveled, soft, irregular, water-oaked areas at or near the torus end (fig. 4, *A*). In sectioned mature fruit internal macroscopic symptoms were evident as a more or less creamy liquefied disorganization of the affected areas. The creamy-white strands of bacterial masses were traced through the placental region and locular cavity directly to the fleshy covering of the seeds. Size of seed was much reduced and the number of aborted and immature seeds was greatly increased in many cases (fig. 4, *B*). In ripe severely infected fruit the entire inner pulp might be completely liquefied but held intact by the shriveled skin.

RELATION OF THE BACTERIA TO THE HOST TISSUE

The movement and distribution of the ring rot organism in the tissues of the tomato plant and fruit were ascertained primarily by microscopic examination of stained serial sections, and in most cases paralleled by stained smears and isolations from similar portions of the host. Systemic invasion of the stem tissues of the tomato occurred

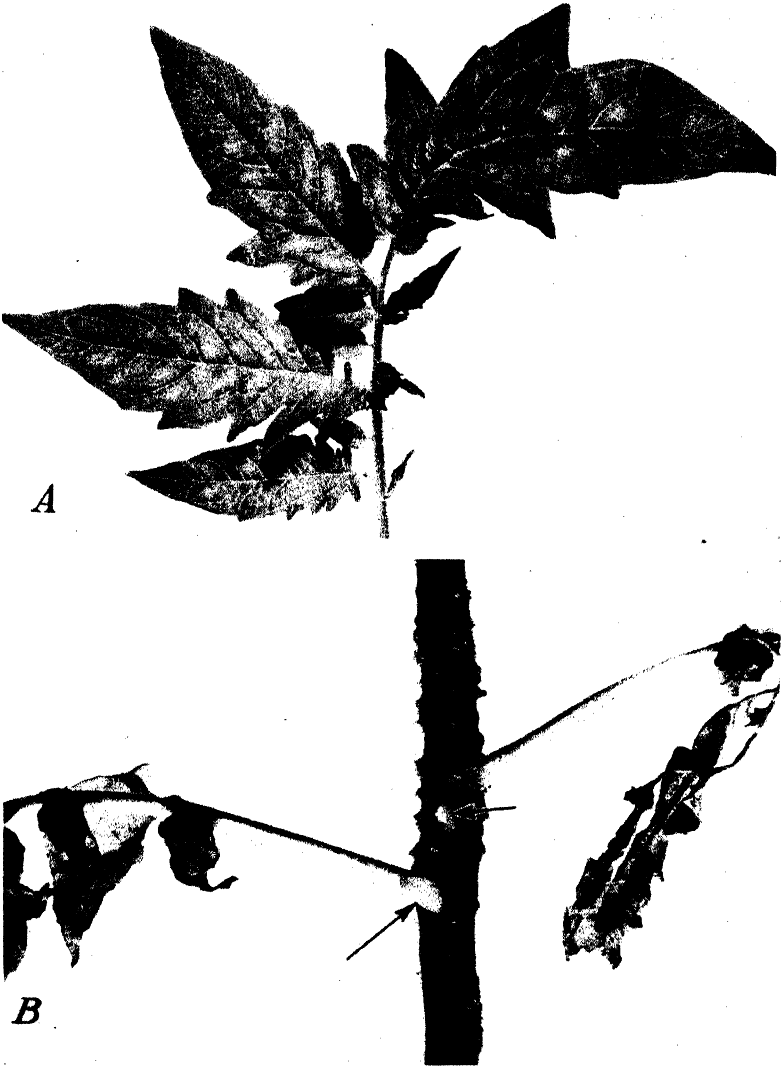


FIGURE 2.—*A*, Unilateral leaf development resulting from systemic invasion by the ring rot organism of one side of petiole, rachis, and midrib of an upper leaf of a young tomato plant. *B*, Exudation of wet, glistening, creamy-white, sticky bacterial masses near leaf nodes following break-down of cortical tissues of a severely infected tomato stem.

largely in the vascular spiral and annular vessels of the xylem elements; the adjacent pitted vessels were less frequently invaded (pl. 1, *A*, *B*). Immature xylem elements and cells of the cambium were only occasionally infected. The outer and inner phloem elements were rarely invaded by the bacteria except in complete break-down of the vascular tissues. Bacteria were not observed in the ray tissue of the older stems.

Seldom were bacteria found in the parenchymatous tissues adjacent to invaded xylem except following tissue rupture near the point of inoculation or at points in the invaded stem where xylem vessels became thickly populated with bacteria and enzymic dissolution of the thinner, weaker-walled xylem cells took place. In these cases small lysigenous cavities in the parenchymatous tissues were formed which were filled with bacteria, and thickened cell walls could be seen lying free within the cavities. In the dissolution of the nonlignified cell walls the bacteria progressed only slightly from the parenchyma cavities into the surrounding intercellular spaces.

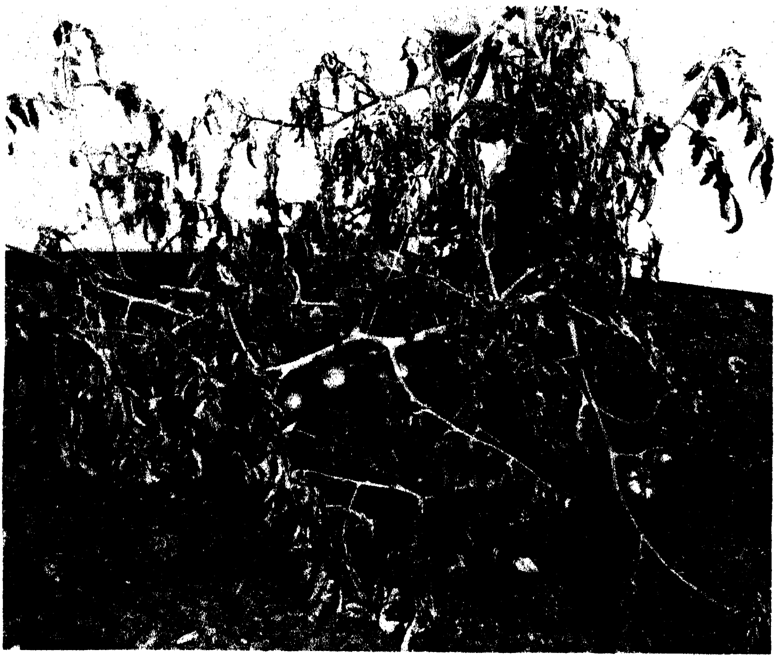


FIGURE 3.—Bonny Best tomato plant, growing in the field, systemically infected with the ring rot organism, showing stunting, irregular wilting, leaf chlorosis and very light fruit set.

Cells of the xylem elements in systemically infected stem tissues did not show disintegration, break-down, or increase in lignification. The transformation of adjacent parenchyma tissues into sclereids was not seen. However, thinner, weaker xylem walls were found in many cases, indicating enzymatic action in advance of the bacteria. This solvent action was also seen in many cases in advance of the bacteria in the intercellular spaces and out from the small lysigenous cavities in the parenchymatous tissues.

In the tissues of the petiole, rachis, and stalk of the leaf, the progressive movement of the ring rot bacteria into the xylem elements was

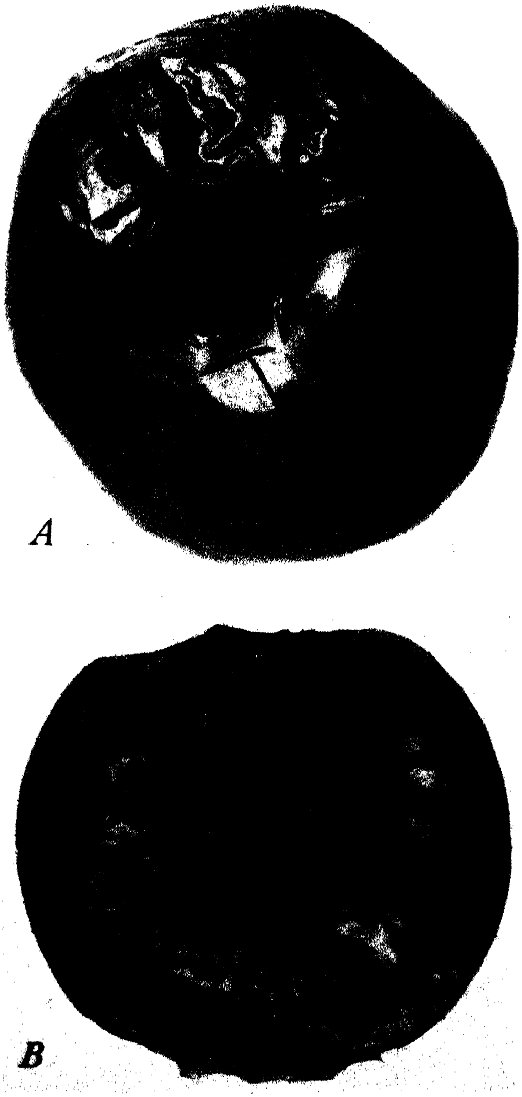


FIGURE 4.—Systemically infected ripe tomato fruits from a diseased plant. *A*, External symptoms on the fruit appearing as irregular, soft, water-soaked areas near the torus end. *B*, Longisection of fruit showing (*a*) bacterial masses in the vascular elements of the placental region appearing as creamy-white strands; (*b*) bacterial aggregates in disintegrated tissues in the locular cavity; (*c*) aborted seeds.

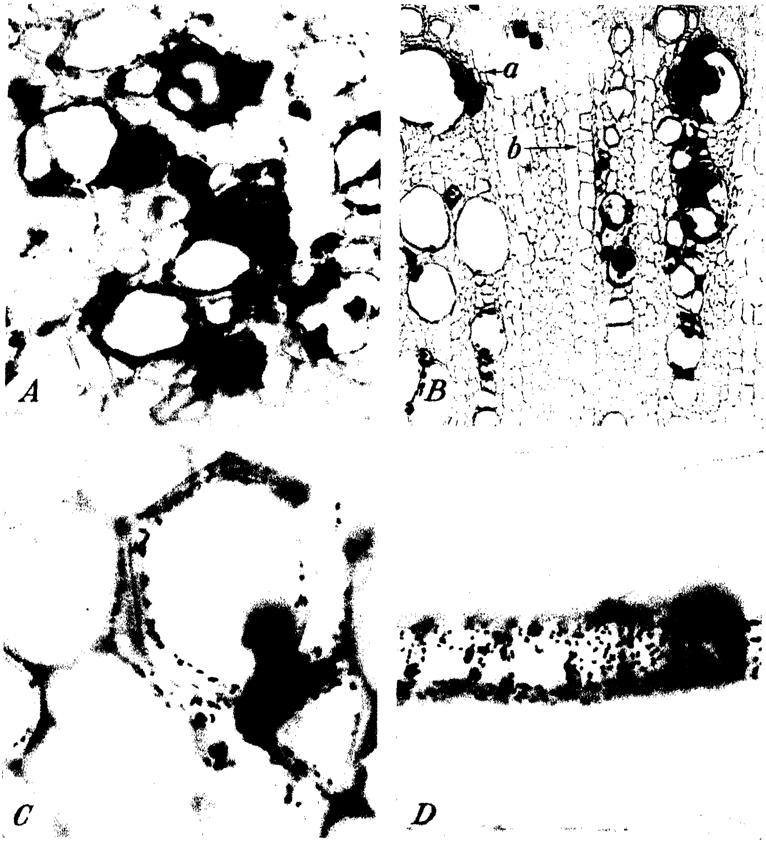
traced from the infected vascular elements of the stem, which in turn gave rise in the petiole to the median or lateral traces. In some cases, however, the vascular tissue of the petiole was invaded via the intercellular spaces of the parenchyma when the same tissues in the stem were similarly affected. Infection of the petiole and rachis was, as in the tissues of the stem, restricted to the spiral and annular elements of the xylem vessels (pl. 1, *C*). Transections of invaded petiolule tissues showed the ring rot bacteria present in the xylem vessels (pl. 1, *D*), and to a slight extent in the spaces between the small parenchyma cells about the heavily infected elements. Cells of the endodermallike tissue, in addition to the xylem elements of infected petioles, were also invaded (pl. 2, *A*) while adjacent cortical and pericycle cells remained free of bacteria. In the latter region, the bacteria were present in relatively smaller numbers than in the corresponding tissue of the petiole. In transections of infected leaflets the organism was seen to be confined entirely to the xylem vessels, many of which were completely filled with bacteria (pl. 2, *B*). In the areas of the leaf blade where the spiral elements of the lateral and branch veins were heavily infested, the cells of the spongy parenchyma were seen to have large numbers of bacteria adhering to their outer walls.

It was found by numerous stained smears, repeated isolations, and the examination of serial histological sections that the systemic advance of the organism in the underground adventitious root tissues of infected tomato plants was somewhat less rapid than in stem and petiole; the difference, however, was not great. The movement of the bacteria in the root tissues was restricted as in the stem to the xylem vessels (pl. 1, *A*). In advanced stages of root infection the cambium became infected to a slight extent and small cavities in the contiguous parenchyma were also to be found. The spread of the organism into the root cortex was not observed and no sclereids were found in any tissues of the root invaded by the ring rot organism.

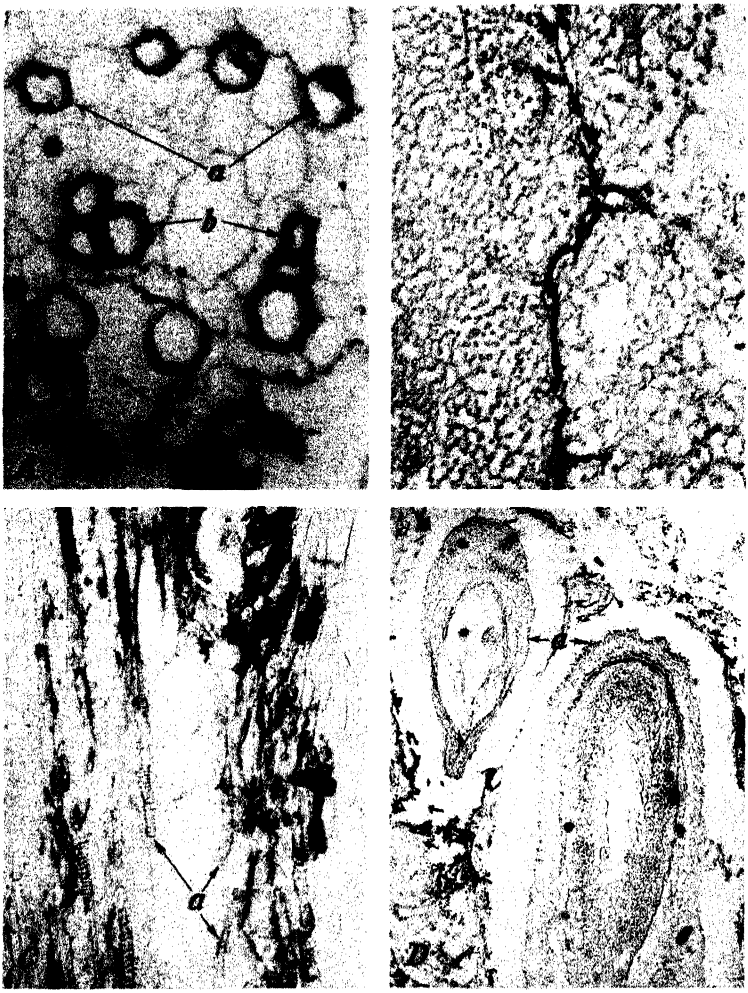
Systemic infection of fruit spurs and fruit of infected plants was first ascertained by stained smears and isolations from portions of the base of the peduncle, the pedicel, the torus, internal fruit tissues, and seed. On examination of histological sections of the different fruit-spur tissues, the ring rot organism was found to be confined entirely to the thick-walled xylem elements. As in the petiole and rachis, unilateral infection of the xylem traces was observed and the path of infection was traced from the traces in the fruit spur to the xylem elements of the stem.

In stained sections of portions of infected mature fruit the ring rot bacteria were traced from the place of fruit attachment through the spirally thickened tracheae of the fleshy placental region (pl. 2, *C*) and locular cavity, directly to the seeds. In the locular cavity large numbers of aborted seeds surrounded by bacterial masse swere commonly found (pl. 2, *D*). In such cases the cells of the integument of the seed coat do not mature.

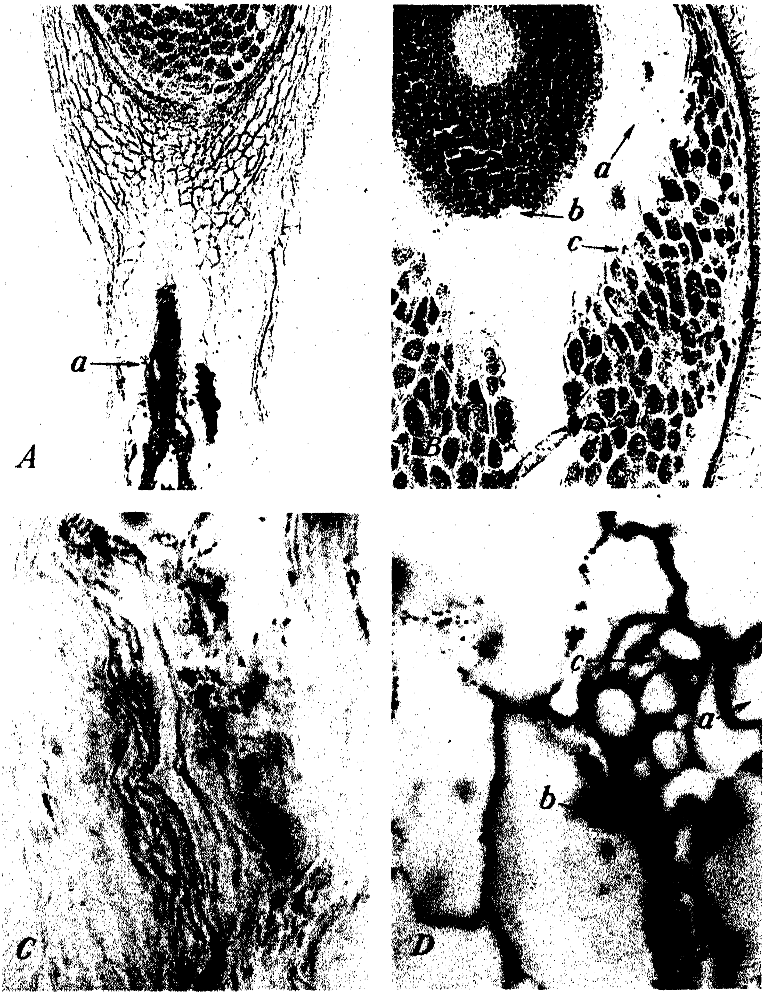
When serial transections and longisections of seeds from systemically infected fruit were examined, bacterial masses were found to be abundant outside the spiny layer of the seed coat and among the hairs formed by the epidermal layer of the seed coat. Large numbers of bacteria were also observed within the spiral vessels in the hilum region and in the funicular tissues remaining attached to the seed



A, Ring rot bacteria in secondary xylem vessels of tomato root. Transection, X 504. *B*, Cross section of portion of stem of mature tomato plant 60 days after inoculation; secondary mature pitted xylem next to cambium (*a*) and ray (*b*) tissues showing bacteria only in the large xylem elements. Transection, X 165. *C*, Systemically infected xylem vessels of the petiole with bacteria also in parenchyma tissue as a result of movement through disintegrated tracheal walls. Transection X 1429. *D*, Spiral vessels of petiolule with abundant bacteria. Transection, X 1142.



A, Transection of infected portion of a tomato petiole showing masses of bacteria in endodermallike cells (*a*) and xylem elements (*b*) and large lysigenous cavities in adjacent parenchmatous tissue (*c*). X 504. *B*, Portion of longisection of lamina of leaf with abundant bacteria in xylem spiral elements of small branch veins. X 160. *C*, Longisection of systemically infected mature tomato fruit showing spiral thickenings of the tracheae (*a*) of the fleshy placental region lying free amid cell debris and bacterial masses; such infected bundles appear macroscopically as cream-colored streaks in severely infected fruit. X 79. *D*, Aborted seeds (*a*) surrounded by bacterial masses in the placental region and lysigenous cavities, in mature infected fruit. Size of seeds is reduced and the number of aborted seeds greatly increased. X 79.



Longisections of tomato seeds (*A* and *B*) from systemically infected fruit and seedlings (*C* and *D*) grown from seed of infected fruit. *A*, Immature seed somewhat aborted, showing portion of the seed and funiculus of the seed; vascular elements of the funiculus including xylem elements and contiguous tissues are destroyed by the ring rot bacteria (*a*). X 125. *B*, Mature seed with bacteria within the seed in the micropylar region (*a*) between the embryo (*b*) and endosperm (*c*). X 125. *C*, Ring rot bacteria in metaxylem elements of hypocotyl tissue of a young seedling grown from infected seed. X 1142. *D*, Stem tissue of young plant grown from infected seed, showing bacteria in pith parenchyma (*a*) and intercellular spaces (*b*); the inner phloem cells (*c*) are not invaded. X 1070.

(pl. 3, *A*). In many cases the bacteria had invaded the parenchyma of the middle layer of the seed coat, and also the region between the integument and the endosperm. Invasion of the micropylar region of the seed between the embryo and endosperm was also observed (pl. 3, *B*).

SEED TRANSMISSION

Evidence has been presented in the histological studies that the bacterial ring rot organism will invade the seed of systematically infected tomato fruit and become established in the spiral vessels in the funiculus of the seed, in the micropylar region between embryo and endosperm within the seed, and on the seed in the roughened integument or seed coat. The viability of the bacteria on the seed coat and within the seed has been proved by culture methods and inoculation.

Seed transmission tests were made with seed harvested from mature field-grown systematically infected fruit. Plantings were made in flats in steamed soil and the seed covered with washed steamed sand. All seeds planted were treated for external contamination with 1–1,000 mercuric chloride, for 3 minutes and rinsed in several changes of sterile water. Plantings were made in the greenhouse at temperatures between 24° to 26° C., which are the most favorable for germination and emergence of tomato seed. The planted flats were watered in such a way as to avoid spattering or splashing the emerging seedlings. Check plantings with seed harvested from known healthy mature fruit from a separate field planting were included. The first plantings were made with seed stored at room temperature for a period of 1 month after harvest and the second plantings were made with seed held 6 months in storage. A total of 30 lots of seed were harvested from infected fruit for the seed transmission tests (table 1).

TABLE 1.—*Emergence of seed lots from infected and healthy fruit, 15 days after planting; 35 seeds in each planting*

Fruit No.	Emergence		Fruit No.	Emergence	
	First planting	Second planting		First planting	Second planting
	Number	*Number*		*Number*	*Number*
1	19	24	19	16	21
2	27	31	20	8	12
3	31	28	21	27	29
4	7	9	22	20	14
5	20	16	23	12	17
[1] 6	32	31	[1] 24	31	33
7	[2] 26	29	25	12	9
8	[2] 21	16	26	24	19
9	[2] 23	20	27	30	26
10	[2] 7	9	28	9	17
11	[2] 8	12	29	23	16
[1] 12	[2] 30	31	[1] 30	34	35
13	25	21	31	26	29
14	14	16	32	15	27
15	27	21	33	26	21
16	29	28	34	19	13
17	24	27	35	21	14
[1] 18	34	32	[1] 36	32	34

[1] Healthy fruit.
[2] Figure 5.

Two weeks after planting an examination of the seedling flats showed a marked difference in percentage of germinability and uniformity of growth of the seedlings in the respective lots (fig. 5). In the emergence of seed harvested from infected fruit the seed coat invariably was carried up above ground. This was very much less evident in seedlings growth from seed of healthy fruit. As the seedlings from seed of infected fruit emerged the curved hypocotyl tissues of the young seedlings were in direct contact with the hilum tissues of the seed coat, and in many cases after emergence the apex of the immature and deformed cotyledonary leaves was held together by the seed coat for some time (fig. 6, *B*, *C*, and *D*).

In addition to histological sections (pl. 3, *C* and *D*), numerous smears made from a large number of emerging seed coats, seedling cotyledons, hypocotyl and stems tissues and stained with Gram's stain showed the ring rot organism to be present in great numbers. Isolations from infected seedling material were identified by the Gram's stain test as well as by inoculations to young Triumph potatoes and to tomato seedlings.

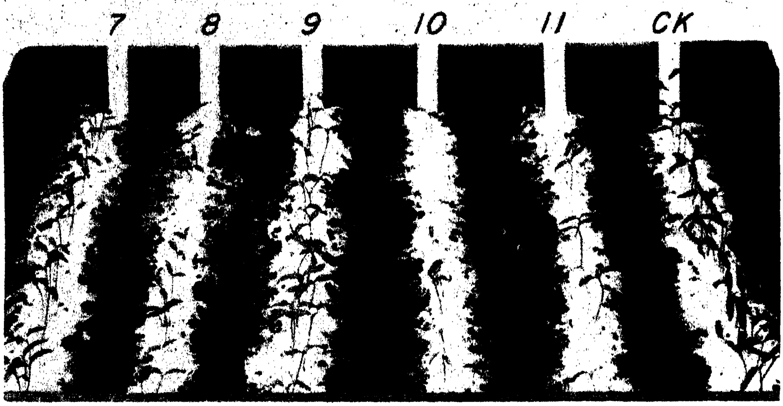


FIGURE 5.—Plantings of tomato seeds harvested from mature infected fruits (first planting Nos. 7 to 11 in table 1) showing various percentages of nonemergence. The last row on the right was planted with seed from healthy mature fruit.

TRANSMISSION THROUGH INOCULATED SOIL

All soil inoculation tests were conducted in an aerated mixture of 3 parts of greenhouse composted soil to 1 part of sand autoclaved in 8-inch clay pots. A portion of the prepared soil was inoculated by thoroughly incorporating a water suspension (100 cc.) of 7-day-old pure cultures of *Corynebacterium sepedonicum* into each 8-inch pot used. Young, healthy tomato transplants were set directly in the freshly inoculated soil. In two series of 40 pots each held in the greenhouse at about 24° C., all plants showed distinct apical dark-green coloration and flaccidity in about 9 days after transplanting. This condition was followed by typical stunting of the plants with shriveling, chlorosis, and necrosis of the lower leaves and unilateral wilting of the apical leaves. All control plants in uninoculated soil to which only sterile water was added, remained healthy.

Histological examination of longisections of numerous tomato roots removed after being exposed to the inoculated soil for 5 days showed very definitely that the avenue of root infection was greatest by far through the wounded root tissues; few cases were observed in which infection took place through what appeared to be uninjured root cortex.

Inoculated soil held in the greenhouse at 20° to 22° C. in a moist condition in flats for 8 weeks and planted to young tomato plants gave only slight infection, 7 affected plants appearing out of 437; no infection occurred when inoculated soil was planted after being held for 12 weeks. When seeds were planted in inoculated soil that had been held in 12-inch pots over winter in the filed, no ring rot infection resulted.

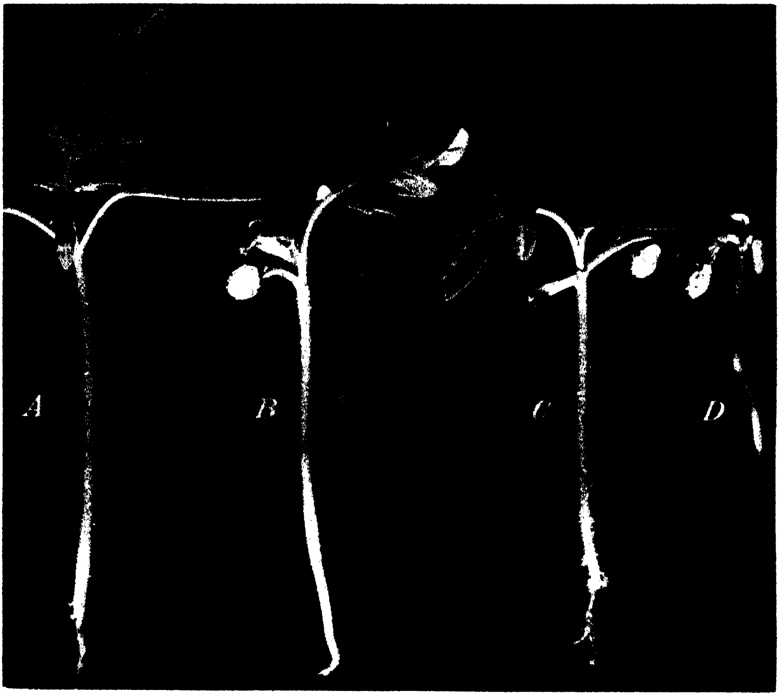


FIGURE 6.—Systemic invasion of tomato seedlings from infected seed. *A*, Seedling from noninfected seed. *B, C, D,* seedlings grown from seed collected from mature systemically infected fruit. Stunted and distorted cotyledonary and primary leaves are to be noted and also the adherence of the seed coat to the cotyledons.

When inoculum (300 cc. water suspension) was applied to the soil surface around healthy young undisturbed tomato seedlings of transplanting age growing in flats, infection rarely resulted. Out of 720 seedlings treated in this manner, only 6 became infected; the bacterial ring rot organism was recovered from those seedlings.

These results indicate that although infection through the wounded root system of tomatoes occurs readily in inoculated soils, the organism does not persist long in the soil. However, when bacterial ring rot has occurred in a field planting of potatoes, the immediate replanting of this ground to tomatoes is not advisable. Moreover, the possible transmission of the disease to young tomato transplants from ring rot infected potato tubers and volunteer potato plants is obvious.

REACTION OF TOMATO VARIETIES TO CORYNEBACTERIUM SEPEDONICUM

To ascertain symptom differences between tomato varieties artificially inoculated with a pure culture of the bacterial ring rot organism, tests involving 70 plants each of 22 commercial varieties and the currant tomato (*L. pimpinellifolium*) were conducted in the greenhouse. Data on severity of symptoms were taken at two phases: (1) at 10 days after inoculation, in the early wilting and dull-green apical leaflet coloration phase, and (2) at 30 days, as the necrotic to moribund phase became pronounced. As recorded in table 2, the following varieties showed the severest early symptoms: Bonny Best, Early Rutgers, Master Marglobe, Morse's Special, Early No. 498, Pan America, Supreme Marglobe, Texas Early, Victor, and Yellow Pear. Moderate early symptoms were expressed by Bounty, Chicago, Del Monte, Early Baltimore, Early Prolific, J. T. D., Marglobe, Pritchard Scarlet Topper, Rutgers, and Stokesdale. John Baer, Pritchard, Riverside, and the currant tomato exhibited slight, somewhat delayed, early-phase symptoms. In the later necrotic to moribund phase all varieties continued to respond and show symptom differences as expressed in the early phase, with the exception of Morse's Special Early No. 498 and Texas Early. These two varieties showed only slight necrosis even after 60 days, although in the early phase wilting was severe. John Baer, Pritchard, Riverside, and the currant tomato exhibited the greatest tolerance; Bonny Best, Early Rutgers, Master Marglobe, Pan America, Supreme Marglobe, and Yellow Pear, were the most susceptible and showed the severest symptoms.

TABLE 2.—*Severity of symptoms in tomato varieties artificially inoculated with Corynebacterium sepedonicum* [1]

Variety	Severity of infection after 10 days (early phase)	Severity of infection after 30 days (late phase)	Variety	Severity of infection after 10 days (early phase)	Severity of infection after 30 days (late phase)
Bonny Best	3	3	Morse's Special Early No. 498	3	1
Bounty	2	3	Pan America	3	3
Chicago	2	2	Pritchard	1	1
Currant	1	1	Pritchard Scarlet Topper	2	2
Del Monte	2	2	Riverside	1	1
Early Baltimore	2	2	Rutgers	2	2
Early Prolific	2	2	Stokesdale	2	3
Early Rutgers	3	3	Supreme Marglobe	3	3
John Baer	1	1	Texas Early	3	1
J. T. D	2	2	Victor	3	3
Marglobe	2	2	Yellow Pear	3	3
Master Marglobe	3	3			

[1] Symptoms recorded on 70 plants of each variety as 1, slight; 2, moderate; and 3, severe.

OTHER SOLANACEOUS HOSTS

Needle inoculations with the bacterial ring rot organism produced severe wilt and vascular infection in all common garden eggplant (*Solanum melongena* L.) varieties tested and also in the wild spiny scarlet eggplant (*S. integrifolium* Poir.).

A severe flaccid condition of the upper leaves with irregular marginal and interveinal bleaching and retarded leaf expansion was apparent in the inoculated eggplants in from 10 to 12 days. As the systemic infection progressed a conspicuous stunting with retarded and unilateral development of the invaded leaf tissues developed, causing a curving of the leaves. This condition was soon followed by a permanent leaf wilting, bronzing, shriveling, cessation of terminal growth and premature death of the entire plant (fig. 7, *A*). No external stem symptoms were apparent in the inoculated test plants; however, a distinct, creamy bacterial ooze was evident in the vascular tissues on cross section of the stems of infected plants. Histological examination of infected stem tissues shows that the bacteria progress through the xylem elements.

The following commercial eggplant varieties were found to be very susceptible to the bacterial ring rot organism and to exhibit severe progressive systemic symptoms: Black Beauty, Black Bountiful, Black King, Garden Prolific, New Hampshire Hybrid, New York Improved, and Extra Early Dwarf Purple. The causal bacteria were reisolated from each of the varieties and tested in all cases by inoculations to young Triumph potatoes and to tomato seedlings.

In the scarlet eggplant (*Solanum integrifolium*), early systemic symptoms following needle inoculation were evident in from 15 to 18 days as slight flaccidity of the upper leaves. On subsequent days a retarded and unilateral growth of the systemically infected lower leaves developed, the malformed portion at first being a very much darker green and gradually changing to interveinal bleaching, approaching chlorosis. As the chlorotic condition increased very often a well marked, progressive, irregular dendritic necrosis of the branch and anastomosing leaf veins appeared (fig. 8). The tissues gradually became dry and brittle, the leaves died prematurely, and terminal growth ceased.

In repeated tests no symptoms developed, and the ring rot organism was not recovered from inoculated test plants of the following Solanaceae:

Atropa belladonna L.
Browallia americana L. var. Speciosa Major.
Capsicum annuum L. (pepper var. Ruby King).
Datura metel L.
Datura meteloides DC.
Datura stramonium L. (jimsonweed).
Hyoscyamus niger L. (black henbane).
Lycium halimifolium Mill. (matrimony-vine).
Nicandra physalodes Pc (Lr.)s. (apple-of-Peru).
Nicotiana acuminata Hook.
Nicotiana angustifolia Ruiz and Pav.
Nicotiana bigelovii S.Wats.
Nicotiana chinensis Fisch.
Nicotiana glutinosa L.
Nicotiana longiflora Cav.
Nicotiana multivalvis Lindl.
Nicotiana quadrivalvis Pursh.

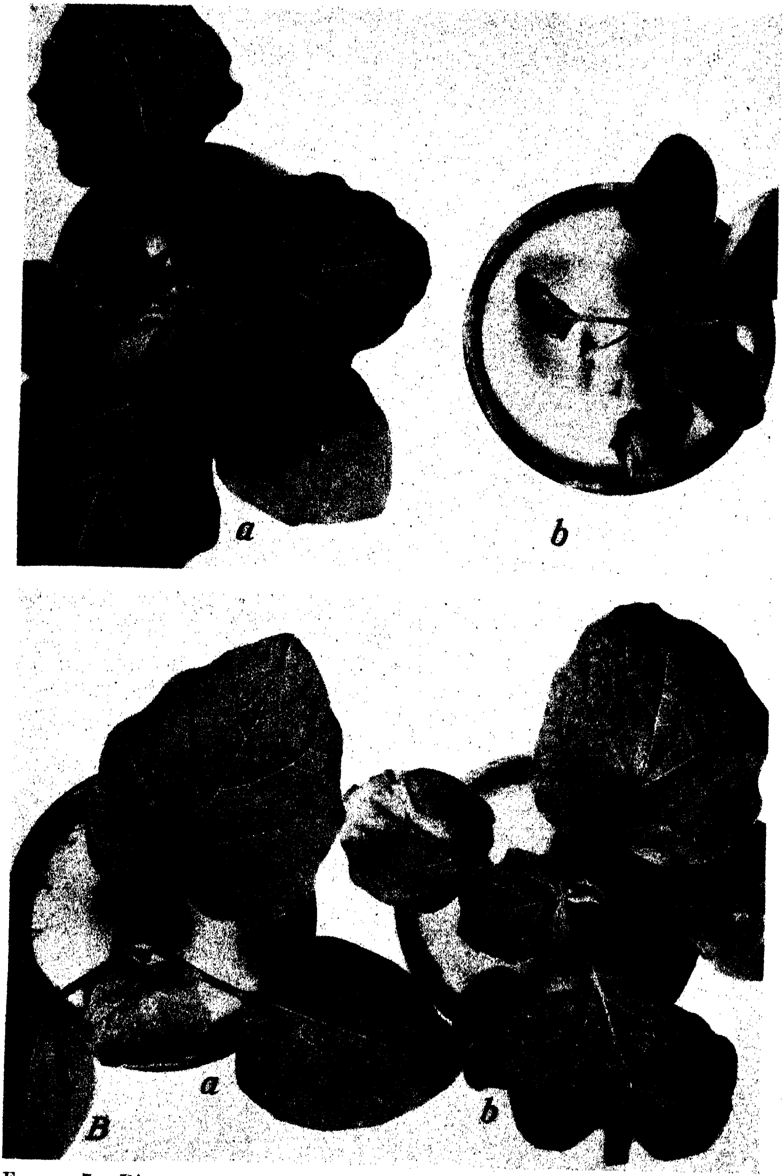


FIGURE 7.—Ring rot on eggplant, photographed 25 days after inoculation, plants grown in the same environment. *A*, Black Beauty variety: *a*, Uninoculated control, *b* inoculated plant, showing extreme stunting, unilateral development of leaves and entire plant, chlorosis, and cessation of terminal growth. *B*, Puerto Rican Beauty variety: *a*, Uninoculated plant; *b*, inoculated plant, with only slight interveinal and marginal bleaching and no wilting of lower leaves.

Nicotiana repanda Willd.
Nicotiana rustica L.
Nicotiana rustica L. var. *brasilia* Schrank.
Nicotiana rustica L. var. *humilis*.
Nicotiana sanderae Sander.
Nicotiana sylvestris Speg. and Comes.
Nicotiana tabacum L. (tobacco var. Connecticut Havana No. 38).
Nierembergia hippomanica Miers var. Purple Robe.
Petunia violacea Lindl. var. Violet Gem.
Physalis aequata Jacq. f.
Physalis heterophylla Nees (clammy groundcherry).
Physalis heterophylla var. *ambigua* (Gray) Rydb.
Physalis lanceolata Michx. (prairie groundcherry).
Physalis longifolia Nutt.
Physalis virginiana Mill.
Salpiglossis sinuata Ruiz and Pav. var. Chamois.

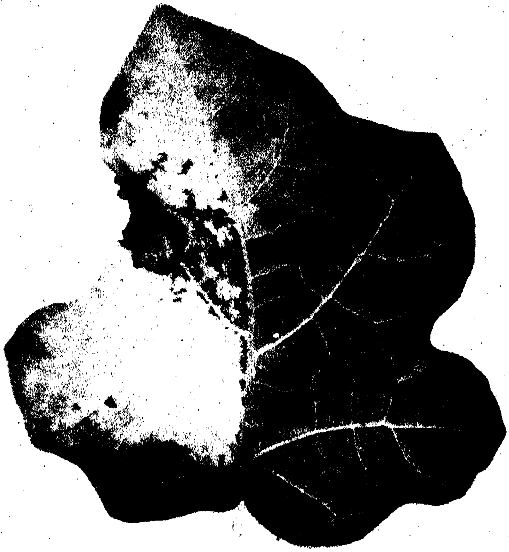


FIGURE 8.—Ring rot in scarlet eggplant (*Solanum integrifolium*). Distortion, bleaching, and dendritic necrosis on leaf from systemically infected plant.

Schizanthus wisetonensis Hort. var. Giant Blotched.
Solanum carolinense L. (horsenettle).
Solanum dulcamara L. (bittersweet).
Solanum nigrum L. (black nightshade).
Solanum pseudo-capsicum L. (Jerusalem-cherry).
Solanum rostratum Dunal (buffalo bur).
Solanum triflorum Nutt.

VARIETAL RESISTANCE IN EGGPLANT

Two varieties of eggplant recently developed by Roque and Adsuar,[7] at the Agricultural Experiment Station of the University of Puerto Rico at Rio Piedras were secured for testing in a ring rot inoculation series along with the very susceptible American Black Beauty. The new varieties were Puerto Rican Beauty, a dark purple Black

---

[7] ROQUE, A., and ADSUAR, J. THE DEVELOPMENT OF NEW VARIETIES OF EGGPLANT RESISTANT TO BACTERIAL WILT. Puerto Rico Univ. (Rio Piedras) Agr. Expt. Sta. Ann. Rpt. 1937–38: 44–45. 1939.

Beauty type of fruit of excellent commercial qualities suited for the export market, and an unnamed selection (E–12), a slender pink-fruited type used for local market. They were developed for resistance to the very serious and destructive soil-borne bacterial disease of the potato and other Solanaceae, bacterial wilt or brown rot (*Bacterium solanacearum* E. F. S.).

In repeated inoculation tests with the bacterial ring rot organism, the two Puerto Rican varieties exhibited a very marked resistance to *Corynebacterium sepedonicum* when compared to the commercial American Black Beauty. The very mild infection that resulted in the inoculated Puerto Rican varieties was expressed by a slight, almost obscure, bleaching of small interveinal and marginal leaf areas in leaves near the point of inoculation, with very little flaccidity, loss of firmness, or wilting of these leaves (fig. 7, *B*). Symptoms of progressive systemic invasion were not observed after the slightly infected lower leaves sloughed. Inoculated test plants of both varieties on being held for long periods in the greenhouse at temperatures favorable for infection showed no further signs of systemic invasion. Neither variety showed any signs of infection on being transplanted into freshly inoculated soil or when inoculum as a suspension was introduced into the soil around the transplants. Inoculated plants on being transplanted to the field showed no wilt symptoms and produced fruit of good quality.

It is important to note that the two varieties of eggplant developed for resistance to a very severe and destructive soil-born bacterial wilt (brown rot) disease also carried a very high degree of resistance to infection by another unrelated bacterial disease of the potato. The two organisms are quite different in their salient physiological and morphological characteristics. *Bacterium solanacearum* is Gram-negative and motile by a single polar flagellum whereas *Corynebacterium sepedonicum* is Gram-positive and nonmotile. The significance of resistance in the Solanaceae to the bacterial ring-rot organism brought out in these preliminary studies is clearly evident.

DISCUSSION

The bacterium (*Corynebacterium sepedonicum*) discussed in this paper has been widespread in the important potato and truck-growing sections of Wisconsin and in recent years has been responsible in many instances for losses in the potato crop. Although the disease has been reported as occurring naturally in the field only on the potato, it is obvious from the results of this investigation and that of other workers, that the bacteria are not specific to this host.

In all commercial varieties of tomato inoculated a decided systemic invasion occurred. The organism was well distributed throughout the vascular elements of affected plants and infection resulted in a typical necrotic or moribund stage.

Thus far the ring rot organism has not been found to overwinter in the soil under Wisconsin conditions, but infected potato tubers as well as infected volunteer potato plants have been found under natural conditions in the field. Ring rot-infected volunteer potato plants may well serve as a very important source of infection for young tomato transplants following a planting of infected potatoes. The planting of tomatoes on fields previously cropped to potatoes that carried even a trace of ring rot the previous season is not considered safe because of the susceptibility of tomatoes to *Corynebacterium sepedonicum*. Every practicable method of sanitation should be considered and practiced not only in tomato culture but with potato production as well.

The ring rot bacteria are carried on and in the seeds of tomato and in this way may play an active part in the persistence and spread of the causal organism.

In the tissues of the tomato plant and fruit the ring rot bacteria are restricted to the vascular spiral and annular vessels of the xylem; the phloem elements are rarely invaded. The parenchymatous tissues adjacent to the xylem are seldom infected although, when invaded, tissue break-down occurs with the formation of small lysigenous cavities. In contrast, in the tomato canker disease (*Corynebacterium michiganense* (E. F. S.) Jensen) the progressive movement of the organism is chiefly in the phloem tissue, and as a result of outward movement the adjacent meristematic tissues are corroded into extensive cavities. In the tomato brown rot disease (*Bacterium solanacearum E. F. S.*) the organism is primarily a parenchyma-vascular parasite, migrating to surrounding tissues from the xylem, causing extensive tissue disintegration and the formation of large lysigenous cavities in both pith and cortex.

SUMMARY

The ring rot bacterium (*Corynebacterium sepedonicum*) may readily cause severe wilting and death of tomato plants. Detailed histological and reisolation studies have shown that the organism invades the plants systemically through the xylem and seldom affects the adjacent phloem and parenchyma in the initial stages of invasion.

The intercellular spaces of younger xylem tissues show only slight enzymatic action in advance of the bacteria; lignification and the formation of sclereids was not observed.

In infected mature fruit the ring rot organism can be traced from the invaded place of fruit attachment through the tracheae in the placental region and locular cavity directly to the funiculus of the seed. In the seed, the bacteria were identified between the integument and endosperm in the micropylar region between embryo and endosperm. Seed transmission has been demonstrated.

All tomato varieties tested were found to be susceptible although some differences in tolerance were observed.

Infection occurred in all tomato transplants when planted in soil recently infested with suspensions of bacteria. No infection resulted on planting tomatoes in inoculated soil that had been held over winter in the field.

All varieties of the common garden eggplant were found to be susceptible; the annual wild weed, the scarlet eggplant (*Solanum integrifolium*), is also susceptible.

Puerto Rican Beauty and selection E–12, two varieties of eggplant resistant to *Bacterium solanacearum*, are also resistant to *Corynebacterium sepedonicum*.

ANALYSIS OF VARIATION IN PANICUM VIRGATUM[1]

By ETLAR L. NIELSEN[2]

Associate agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

In the Great Plains region, *Panicum virgatum* L., commonly called switchgrass, is used for grazing, for hay, and for soil-conservation purposes. Because of its economic uses and also because of its wide range of naturally occurring types and its characteristic good seeding habit, switchgrass is considered by some agronomists a desirable forage species.

The recent interest in the expansion and improvement of the grasslands of the United States has resulted in the establishment of a number of forage-plant nurseries in different parts of the country. The wide morphological range of the numerous biotypes assembled in these nurseries as potential breeding stocks is immediately recognized. The study here reported is the result of the writer's efforts to find reliable characters for a logical grouping of the biotypes of *Panicum virgatum* that had been assembled in the grass nursery of the University of Arkansas. It should be recognized that the results reported are an analysis of only the isolates[3] assembled and do not purport to be a critical analysis of *Panicum virgatum* as a whole. They do, however, indicate the complexity of the problem of segregation within this species, of which two varieties and the typical species were recognized by Hitchcock (*18*),[4] who relegated to synonymy other segregates described by other systematists.

MATERIAL AND METHODS

PLANT MATERIAL

Seed and clonal stocks of *Panicum virgatum* were collected or otherwise procured from a number of localities, mostly in the Great Plains region of the United States (table 1). Here the species commonly occurs in association with other tall-grass prairie species. In drier localities it frequents the bottom lands adjoining streams and swales. These seed and clonal stocks were mass-planted in duplicate rows in the grass nursery located on the Agricultural Experiment Station

---

[1] Received for publication March 24, 1943. Most of the work reported in this paper was conducted in the laboratories of the College of Agriculture, University of Arkansas, Fayetteville, Ark. The investigations were completed at Madison, Wis., in cooperation with the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases, U. S. Department of Agriculture. Research paper No. 754, Journal Series, University of Arkansas, published with the approval of the Directors of the Arkansas and the Wisconsin Agricultural Experiment Stations.

[2] The writer acknowledges the cooperation extended in procuring seed of *Panicum virgatum*, particularly by members of the Soil Conservation Service, U. S. Department of Agriculture. National Youth Administration students aided materially in the measurements of the several organs reported upon. The author also acknowledges the suggestions and criticisms made by Dr. C. H. Wadleigh, of the U. S. Regional Salinity Laboratory, Riverside, Calif., and by Dr. D. C. Smith and Dr. J. H. Torrie, of the University of Wisconsin.

[3] In this paper the term "isolate" is used to denote any particular segregate or accession collected.

[4] Italic numbers in parentheses refer to Literature Cited, p. 352.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 8
Oct. 15, 1944
Key No. G-1315

farm, Fayetteville, Ark. The soil of the nursery site, a silt loam of the Newtonia series, is of moderate to low productivity. Plants grown on this site, and others to be mentioned subsequently, comprise the material on which this paper is based. In sampling these rows for the morphological comparisons, the culms were taken at random over the entire length of the duplicate rows.

TABLE 1.—*Sources and chromosome numbers of isolates examined*

Arkansas University accession No.	Chromosone number ($2n$)	Source
1432	18	Chippewa Falls, Wis.
1304	36	Mandan, N. Dak.
345, 345B, 345C, 345D, 1370, 1437, 1596	36	Stillwater, Okla.
1599, 1606	36	Perkins, Okla.
1602	36	Vernon, Tex.
1376, 1411, 1414, 1426, 1429, 1434	36	Chippewa Falls, Wis.
1545	54	North central Arizona.
644	54	Rogers, Ark.
1032	54	Johnson, Ark.
1598	54	Fort Smith, Ark.
1321	54	Greeley, Colo.[1]
1422	54	Chippewa Falls, Wis.
1445	54	Locality unknown.
1047	72	Bentonville, Ark.
1198	72	Canfield, Ark.
966	72	Fayetteville, Ark.
1324, 1326, 1331	72	Greeley, Colo.
1608	72	Amelia, Nebr.
25, 343	72	O'Neil, Nebr.
1329, 1330, 1594	72	Holt County, Nebr.
1305	72	Lincoln, Nebr.
345A, 1500,[2] 1568[2]	72	Stillwater, Okla.
1410, 1413, 1420, 1425	72	Chippewa Falls, Wis.
340, 1322, 1323, 1327	90	Greeley, Colo.[1]
1603	90	Liberal, Kans.
339	90	O'Neil, Nebr.
1328	90	Holt County, Nebr.
1597[2]	90	Perkins, Okla.
1380, 1409, 1416, 1421, 1435	90	Chippewa Falls, Wis.
1325[2]	108	Greeley, Colo.

[1] Original source. Seed used produced in Soil Conservation Service nursery, Lincoln, Nebr.
[2] Perhaps number indicated ±1, 2.

FIXATION AND STAINS

Chromosome numbers were determined in root tips taken from greenhouse-grown plants. These were fixed in a modified LaCour's 2 BD fixative and stained with crystal violet. The drawings of the chromosome complements (see fig. 1, *A–T*) were made with the aid of a camera lucida at a projected magnification of 2,750 diameters.

MORPHOLOGICAL MEASUREMENTS

Most of the morphological measurements reported are from plants grown in nurseries in 1938. Exceptions are those isolates reported in table 1 as taken from the Chippewa River terrace near the municipal water-pumping station at Chippewa Falls, Wis. In these cases the material was cut at the ground level in August 1938. The lack of the fourth-node data, where leaves are indicated as being present, was due to stems having been cut above this node, which was at the ground level. Each isolate thus collected was dried and wrapped separately. Later it was moistened and measured in the laboratories at the University of Arkansas. The measurement of each of the organs considered was made as follows.

The height of the culm, expressed in centimeters, was measured from the ground level to the tip of the panicle. The diameters of the first and fourth nodes were arbitrarily selected for measurement with vernier calipers. Lower nodes were not selected, because their accompanying leaves are frequently in poor condition or because, in some instances, some races of this species have none. The length of the uppermost internode was measured from the lowermost branch of the inflorescence to the first cauline node.

The length of the inflorescence was measured from the base of the lowermost branch of the panicle to the tip of the inflorescence. Likewise, the length of the lowermost branch was measured from the insertion of the branch on the central axis to the tip of its most distal spikelet. Measurements were made with a metric rule.

The length of the mature and ripened spikelet was measured from the base of the glumes to the tip of the sterile lemma. This length and the length of the caryopsis were determined with a vernier caliper.

The length of the blades of the first and fourth leaves below the inflorescence was measured from the ligule to the leaf tip; the width was determined 1 cm. above the ligule in order to overcome the natural curvature near the blade base.

The diameters of the rhizomes were determined from measurements made at the nodes; the length of the internode was measured from one scale scar to the next. The diploid and hexaploid isolates and one octoploid isolate had basal caulicles instead of rhizomes and therefore are not considered in the discussion of rhizomes.

No characters that were subject to variations in the judgment of the observers were considered in this study. The evaluation of such plant characters as color, glabrousness or pilosity, and scabrousness or smoothness are subject to personal opinion and bias and were therefore omitted. There is a wide variation in these qualitative characters in *Panicum virgatum*, but a description of them would add little to the verification of the heterogeneity of this species as expressed by quantitative characters. There was also a wide variation in the pathogenic reactions of the isolates studied. This has been amply shown in a recent paper by Cornelius and Johnston (*9*), wherein isolates assembled at Manhattan, Kans., were considered.

STATISTICAL TREATMENT

For purposes of comparison of the different structures, the standard errors of the mean difference among isolates were calculated and used to derive the corresponding *t* values, whose significance was ascertained from Snedecor's tables (*26*). In the discussion, only differences having odds of 99 : 1 against their being due to random chance are considered as significant.

EXPERIMENTAL RESULTS

CHROMOSOME NUMBER AND MORPHOLOGY

The widespread occurrence of polyploidy in the Gramineae has been indicated in the studies reported by a number of workers (*1*, *2*, *4*, *5*, *6*, *10*, *11*, *19*, *20*, *24*, *25*, *27*, *28*, *29*, *30*), but it was not until recently that polyploidy was known to occur in *Panicum virgatum*. Church (*7*) reported tetraploid and octoploid races of this species in plants grown from seed produced in Kansas and Oklahoma. In the same paper he reported the tetraploid number 36 for *P. virgatum* var. *spissum* Linder. Church's observations concerning the midwestern material have been fully substantiated in the present study. In addition to tetraploid and octoploid isolates, however, diploid, hexaploid, decaploid, and 12-ploid segregates have been found. In this section of the present paper, the observations concerning chromosome number and morphology will be considered briefly, and these numbers will be referred to in the comparisons of the morphological and nuclear characters in the next section.

One diploid isolate having 18 somatic chromosomes was studied (table 1). The chromosomes were about 0.5μ by 2.0μ and were usually rodlike to somewhat U-shaped (fig. 1, *A*).

Seventeen of the isolates studied were tetraploids. Most of the chromosomes examined were rodlike or U-shaped. Considerable variation was observed in the diameters of the chromosomes in complements of several of these isolates. The figures examined had chromosomes 0.3μ to 0.5μ in diameter and 0.7μ to 2.5μ in length (fig. 1, *B*, *C*, *D*). In isolate 345, introduced from a bulk sample into the grass nursery, four plants decidedly different in appearance from other "sister" plants appeared. These were isolated and designated as isolates 345A to 345D, respectively. One of these divergent forms (345A) was found to have 72 chromosomes and will, therefore, be considered in connection with other octoploid isolates.

Seven hexaploid isolates were examined, and the variations in chromosome size of the individual complements were found to be similar to those seen in the tetraploid complex. The smaller members of complements of the hexaploid complex were mostly 0.3μ by 1.0μ; other members of the same complements were as large as 0.5μ by 2.5μ. An examination of figure 1, *E*, *F*, and *G*, shows that in the hexaploid complex the chromosomes vary considerably in size.

Twenty octoploid isolates were examined. There was a tendency for the individual chromosomes of the complements to be small in diameter, usually about 0.3μ. In length they ranged generally from 1.0μ to 1.5μ, but in isolates 343 and 966 chromosomes ranging from 1.0μ to 3.0μ long were observed. In some isolates (1330, for example), nearly all members of the complements were from 1.0μ to 1.5μ long (fig. 1, *H–P*).

In the decaploid complex, 13 isolates were studied. The chromosomes were usually 0.3μ or less in diameter, but in some complements individual members were larger. They were usually rodlike, less frequently somewhat curved, and from 0.7μ to 1.3μ in length (fig. 1, *Q*, *R*, *S*).

One isolate contained 108 chromosomes. The chromosomes were usually short, 1.0μ or less in length, and about 0.2μ to 0.3μ in diameter. There was some variation in chromosome size in this isolate, as there had been in most of the other number groups (fig. 1, *T*).

The chromosomes of the isolates examined were highly variable in length and diameter throughout the entire series. Those of isolates having higher chromosome numbers tended, however, to be somewhat smaller in diameter than those of isolates having lower chromosome numbers. Reference to the sources of the material from which these isolates were obtained gives no indication of regional segregation of

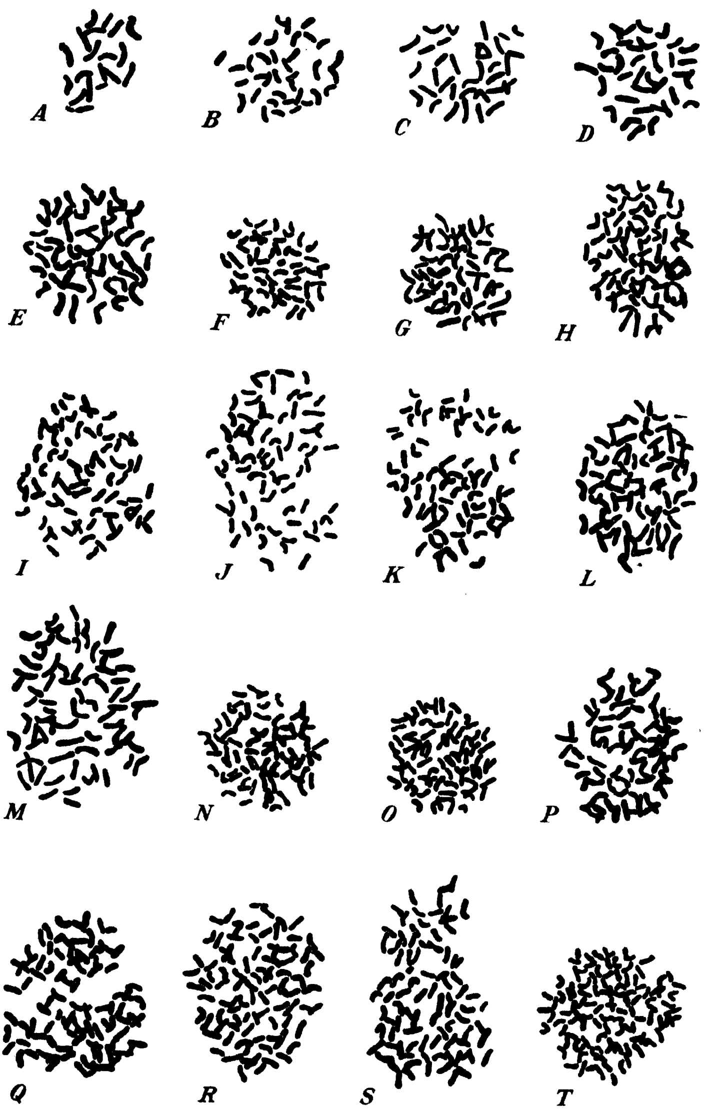


FIGURE 1.—Camera lucida drawings of chromosome complements in somatic tissues. *A*, No. 1432, $2n=18$, Chippewa Falls, Wis.; *B*, No. 345, $2n=36$, Stillwater, Okla.; *C*, No. 345B, $2n=36$, Stillwater; *D*, No. 345C, $2n=36$, Stillwater; *E*, No. 1445, $2n=54$, locality unknown; *F*, No. 644, $2n=54$, Rogers, Ark.; *G*, No. 1321, $2n=54$, Greeley, Colo.; *H*, No. 1198, $2n=72$, Canfield, Ark.; *I*, No. 1331, $2n=72$, Greeley; *J*, No. 1330, $2n=72$, Holt County, Nebr.; *K*, No. 345A, $2n=72$, Stillwater; *L*, No. 25, $2n=72$, O'Neil, Nebr.; *M*, No. 966, $2n=72$, Fayetteville, Ark.; *N*, No. 1413, $2n=72$, Chippewa Falls; *O*, No. 1324, $2n=72$, Greeley; *P*, No. 1608, $2n=72$, Amelia, Nebr.; *Q*, No. 1416, $2n=90$, Chippewa Falls; *R*, No. 1322, $2n=90$, Greeley; *S*, No. 340, $2n=90$, Greeley; *T*, No. 1325, $2n=108$, Greeley. × about 1,900.

type on the basis of chromosome number. Of especial interest is the wide range of chromosome numbers found in the material taken from near Chippewa Falls, Wis. The writer personally collected all of these isolates, and others not yet examined for chromosome number, from an area of not more than 10 acres on the terrace adjoining the Chippewa River, about midway between Chippewa Falls and the city water-pumping station. It is readily conceivable that the many distinct types found in this situation were introduced there through seed in hay fed to horses during logging operations in the early years of this century. If these types were introduced, it may be stated with reasonable certainty that they have successfully established themselves and can be considered as acclimated to Wisconsin weather and soil conditions. It is also possible that these forms have developed through hybridization. If they have arisen as a result of hybridization, it is probable that many of the progeny are still in a stage of genetic instability and that new forms are constantly appearing.

GROSS MORPHOLOGY

Individual comparisons of the organs studied will not be discussed, nor will the comparative sizes of these organs be given in the text. The latter may be ascertained for isolates of the several complexes in the regressions (figs. 2–8). This discussion will only indicate the trends found in the several organs considered and the morphological diversity of the population examined.

The comparisons are summarized in table 2, which shows the number of comparisons made and the number of those significantly different at the 1-percent level. The total population of 28 isolates is referred to as the "A" population, "A" comparison, etc., and the 7 Wisconsin isolates as the "W" population, "W" comparison, etc. These are considered in the following manner: (1) The isolates within a given chromosome complex are compared with all other isolates of the same chromosome complex; (2) all isolates of a given chromosome complex are compared with those of all the other chromosome complexes. This treatment of the data makes it easier to ascertain the heterogeneity within and between chromosome complexes in two types of populations: (1) The total population, which includes all the isolates grown near Fayetteville, Ark., and the isolates collected near Chippewa Falls, Wis.; and (2) the Wisconsin isolates separately. The former population comprises a group of isolates gathered from different topographic, geographic, and edaphic situations and grown together on a single site; incorporated with these are the Wisconsin isolates, also grown on a single site. The treatment of the Wisconsin material as a separate unit affords the opportunity to consider a group of isolates grown and collected from a single area under similar edaphic, topographic, and geographic conditions.

Table 2 shows the total number of comparisons of each organ and the number and percentage found significantly different for isolates of the same chromosome complex and for isolates of the different chromosome complexes. Although these do not bear directly upon the problem under discussion, they serve to illustrate the dispersion of the population in relation to the different characters.

The polymorphism of the population of *Panicum virgatum* studied is readily seen in table 2. For more direct comparison, table 3 gives

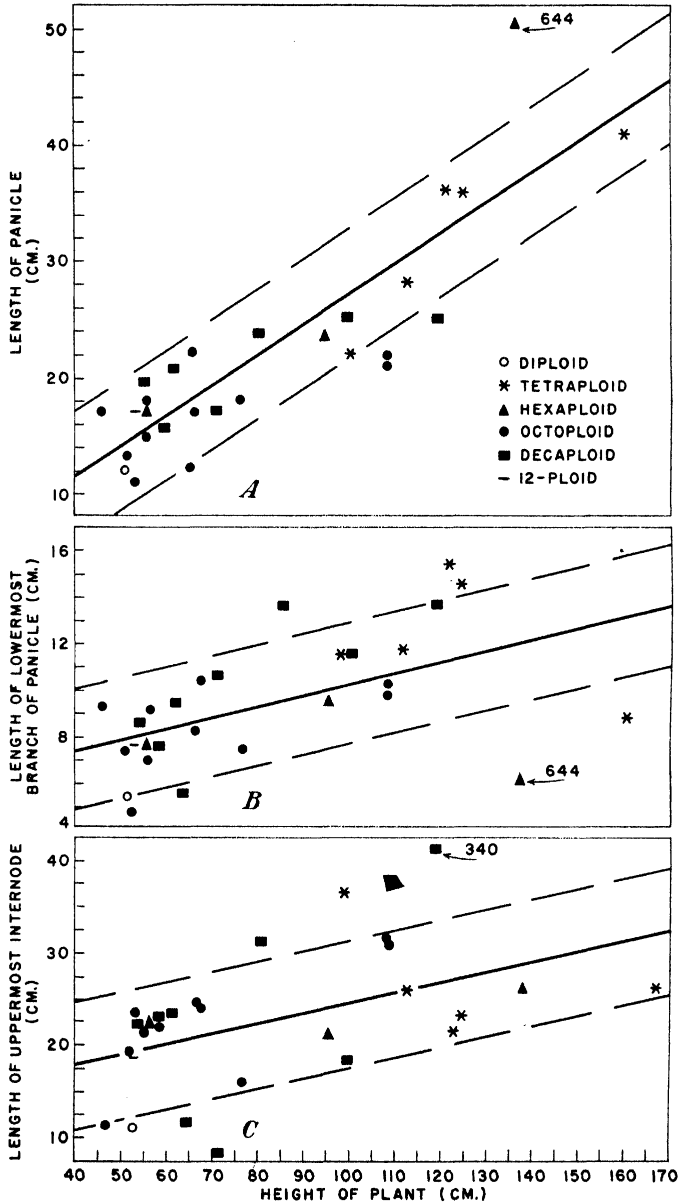


Figure 2.—Calculated regression and standard error of estimate for various characters to height of plant. Actual magnitudes of these characters are plotted under the symbols shown. *A*, Length of panicle; *B*, length of lowermost branch of panicle; *C*, length of uppermost internode.

TABLE 2.—*Number of possible comparisons and number and percentage of comparisons significantly different between isolates of the same chromosome number and between isolates of different chromosome numbers in populations A and W of Panicum virgatum*

Comparison	Plant height				Panicle length				Length lower panicle branch				Length first internode				Length first leaf				Width first leaf				Diameter first node				Length fourth leaf			
	A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]	
2n versus—																																
4n	5	5	1	1	5	5	1	1	5	4	1	1	5	5	1	1	5	5	1	1	5	4	1	0	5	4	1	0	4	4	---	---
6n	3	2	---	---	3	3	---	---	3	1	---	---	3	3	---	---	2	2	---	---	3	2	---	---	3	2	---	---	2	1	---	---
8n	10	4	2	1	10	7	2	2	10	5	2	1	10	9	2	1	10	9	2	2	10	4	2	2	10	5	2	2	10	4	2	2
10n	8	4	3	2	8	7	3	2	8	5	3	2	8	7	3	2	8	7	3	2	8	3	3	1	8	4	3	1	5	4	2	2
12n	1	0	---	---	1	1	---	---	1	0	---	---	1	1	---	---	1	1	---	---	1	0	---	---	1	0	---	---	---	---	---	---
All others	27	15	6	4	27	23	6	5	27	15	6	4	27	25	6	4	26	24	6	5	27	13	6	3	27	15	6	3	21	13	4	4
4n versus—																																
4n	10	7	---	---	10	9	---	---	10	8	---	---	10	5	---	---	10	7	---	---	10	7	---	---	10	7	---	---	6	5	---	---
6n	15	14	---	---	15	13	---	---	15	11	---	---	15	8	---	---	10	6	---	---	15	10	---	---	15	14	---	---	8	6	---	---
8n	50	47	2	2	50	45	2	1	50	32	2	1	50	29	2	2	50	35	2	0	50	42	2	1	50	42	2	0	40	36	---	---
10n	40	36	3	3	40	35	3	2	40	23	3	1	40	27	3	3	40	29	3	1	40	32	3	2	40	30	3	0	20	17	---	---
12n	5	5	---	---	5	5	---	---	5	4	---	---	5	4	---	---	5	3	---	---	5	4	---	---	5	4	---	---	---	---	---	---
All others	115	107	6	6	115	103	6	4	115	74	6	3	115	73	6	6	110	78	6	2	115	92	6	3	115	94	6	0	72	63	---	---
6n versus—																																
6n	3	3	---	---	3	3	---	---	3	1	---	---	3	2	---	---	1	0	---	---	3	2	---	---	3	3	---	---	1	1	---	---
8n	30	25	---	---	30	20	---	---	30	8	---	---	30	15	---	---	20	11	---	---	30	22	---	---	30	22	---	---	20	14	---	---
10n	24	20	---	---	24	15	---	---	24	13	---	---	24	17	---	---	16	10	---	---	24	13	---	---	24	16	---	---	10	7	---	---
12n	3	2	---	---	3	2	---	---	3	0	---	---	3	1	---	---	2	1	---	---	3	2	---	---	3	2	---	---	---	---	---	---
All others	75	63	---	---	75	53	---	---	75	33	---	---	75	44	---	---	50	30	---	---	75	56	---	---	75	56	---	---	40	28	---	---
8n versus—																																
8n	45	29	1	1	45	24	1	0	45	4	1	0	45	28	1	1	45	14	1	0	45	22	1	0	45	19	1	0	45	23	1	0
10n	80	58	6	4	80	45	6	4	80	36	6	3	80	56	6	5	80	39	6	2	80	55	6	4	80	36	6	1	50	33	4	0
12n	10	5	---	---	10	4	---	---	10	3	---	---	10	5	---	---	10	1	---	---	10	5	---	---	10	2	---	---	---	---	---	---
All others	180	139	10	9	180	121	10	7	180	84	10	5	180	114	10	8	170	95	10	4	180	128	10	7	180	107	10	3	120	87	6	2
10n versus—																																
10n	28	20	3	1	28	21	3	3	28	19	3	3	28	25	3	3	28	20	3	2	28	21	3	3	28	17	3	1	10	9	1	0
12n	8	6	---	---	8	4	---	---	8	4	---	---	8	5	---	---	8	3	---	---	8	4	---	---	8	4	---	---	---	---	---	---
All others	160	124	12	9	160	106	12	8	160	81	12	6	160	112	12	10	152	88	12	5	160	107	12	7	160	90	12	2	85	61	6	2
12n versus all others	27	18	---	---	27	16	---	---	27	11	---	---	27	16	---	---	26	9	---	---	27	15	---	---	27	12	---	---	---	---	---	---
Total within complexes	86	59	4	2	86	57	4	3	86	32	4	3	86	60	4	4	84	41	4	2	86	52	4	3	86	46	4	1	62	38	2	0
Percentage differences[2] within complexes	68.6		50.0		66.3		75.0		37.2		75.0		69.8		100.0		48.8		50.0		60.5		75.0		53.5		25.0		61.3		0.0	
Total between complexes	584	466	34	28	584	422	34	24	584	298	34	18	584	384	34	28	534	324	34	16	584	411	34	20	584	374	34	8	338	252	16	8
Percentage differences[2] between complexes	79.8		82.4		72.3		70.6		51.0		52.9		65.8		82.4		60.7		47.1		70.4		58.8		64.0		23.5		74.6		50.0	

Comparison	Width fourth leaf				Diameter fourth node				Length rhizome internode				Diameter rhizome node				Spikelet length				Caryopsis length				Nuclear diameter				Total comparisons and total significantly different				Percentage significantly different	
	A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A	W
2n versus—																																		
4n	4	4			4	4											5	5	1	1	5	5	1	1	5	5	1	1	62	59	10	8	95.2	80.0
6n	3	2			3	2											2	2			2	2			3	2			35	26			74.3	
8n	10	5	2	2	9	3	2	1									10	10	2	2	10	10	2	2	10	8	2	2	129	83	26	22	64.3	84.6
10n	6	4	2	1	6	4	2	2									8	8	3	3	8	8	3	3	8	5	3	2	97	70	36	25	72.2	69.4
12n																	1	1			1	1			1	1			10	6			60.0	
All others	23	15	4	3	22	13	4	3									26	26	6	6	26	26	6	6	27	21	6	5	333	244	72	55	73.3	76.4
4n versus—																																		
4n	6	3			6	4											10	6			10	5			10	5			118	78			66.1	
6n	12	9			12	9											10	6			10	7			15	4			167	117			70.1	
8n	40	36			36	36											50	18	2	1	50	34	2	1	50	24	2	2	616	456	20	11	74.0	55.0
10n	24	19			24	24											40	14	3	1	40	27	3	3	40	25	3	3	468	338	30	19	72.2	63.3
12n																	5	5			5	5			5	5			50	44			88.0	
All others	80	68			76	73											110	48	6	3	110	78	6	5	115	63	6	6	1,363	1,014	60	38	74.4	63.3
6n versus—																																		
6n	3	3			3	3			1	1			1	1			1	1			1	1			3	1			33	26			78.8	
8n	30	29			27	20			18	10			18	13			20	14			20	15			30	15			383	253			66.1	
10n	18	14			18	16			16	11			16	9			16	11			16	11			24	15			294	198			67.3	
12n									2	1			2	1			2	2			2	1			3	3			31	18			58.1	
All others	63	49			60	47			36	22			36	23			50	35			50	35			75	39			910	613			67.4	
8n versus—																																		
8n	45	27	1	0	36	14	1	0	36	26	1	0	36	21	1	0	45	19	1	1	45	32	1	1	45	29	1	0	648	331	15	4	51.1	26.7
10n	60	45	4	3	54	30	4	2	72	42	6	2	72	47	6	2	80	28	6	1	80	44	6	2	80	55	6	6	1,108	649	84	41	58.6	48.8
12n									9	7			9	6			10	9			10	5			10	8			118	60			50.8	
All others	140	110	6	5	126	89	6	3	99	59	6	2	99	66	6	2	170	79	10	4	170	108	10	5	180	110	10	10	2,354	1,496	130	76	63.6	58.5
10n versus—																																		
10n	15	15	1	1	15	11	1	1	28	17	3	2	28	16	3	0	28	8	3	2	28	15	3	1	28	20	3	2	376	254	39	25	67.6	64.1
12n									8	6			8	3			8	7			8	3			8	8			96	57			59.4	
All others	108	82	6	4	102	74	6	4	96	59	6	2	96	59	6	2	152	68	12	5	152	93	12	8	160	108	12	11	2,063	1,312	150	85	63.6	56.7
12n versus all others									19	14			19	10			26	24			26	15			27	25			305	185			60.7	
Total within complexes	69	48	2	1	60	32	2	1	65	44	4	2	65	38	4	0	84	34	4	3	84	53	4	2	86	55	4	2						
Percentage differences[2] within complexes	69.6		50.0		53.3		50.0		67.7		50.0		58.5		0.0		40.5		75.0		63.1		50.0		64.0		50.0							
Total between complexes	414	324	16	12	386	296	16	10	250	154	12	4	250	158	12	4	534	280	34	18	534	355	34	24	584	366	34	32						
Percentage differences[2] between complexes	78.3		75.0		76.7		62.5		61.6		33.3		63.2		33.3		52.4		52.9		66.5		70.6		62.7		94.1							

[1] Number of possible comparisons in first column; number of significantly different comparisons at 1-percent level in second column.

[2] Percentage of statistically significant differences.

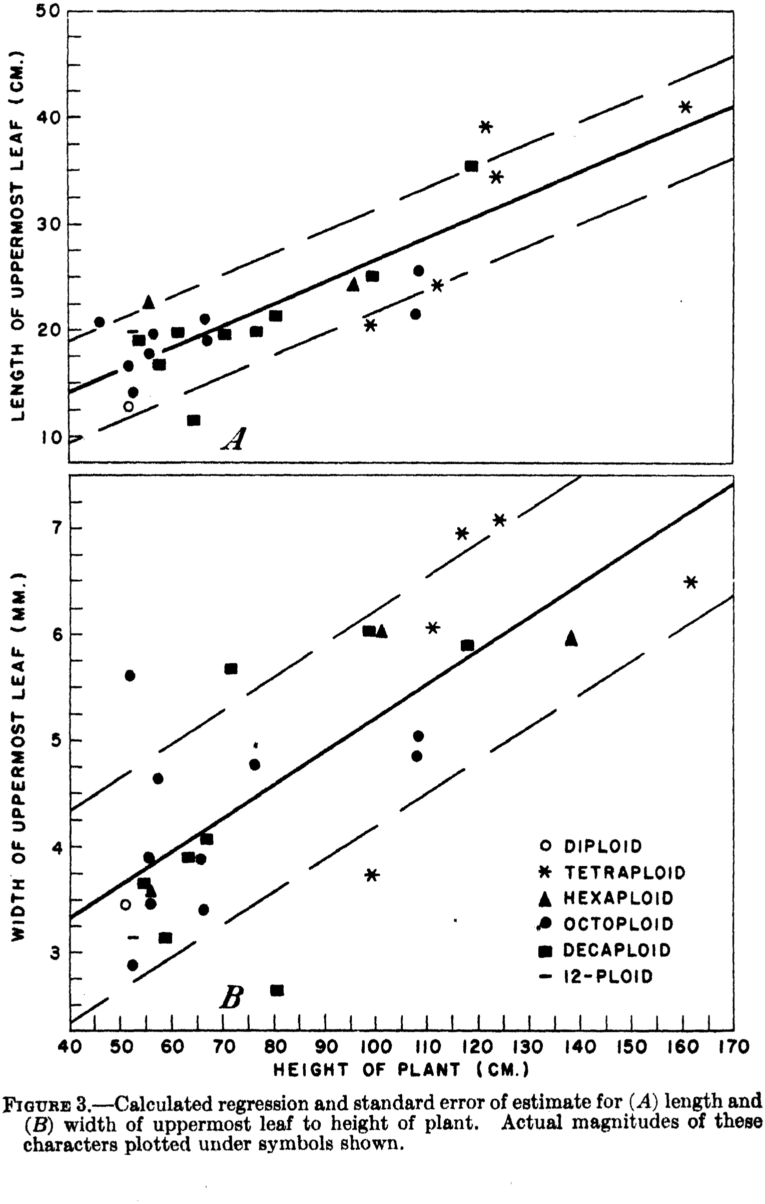


FIGURE 3.—Calculated regression and standard error of estimate for (*A*) length and (*B*) width of uppermost leaf to height of plant. Actual magnitudes of these characters plotted under symbols shown.

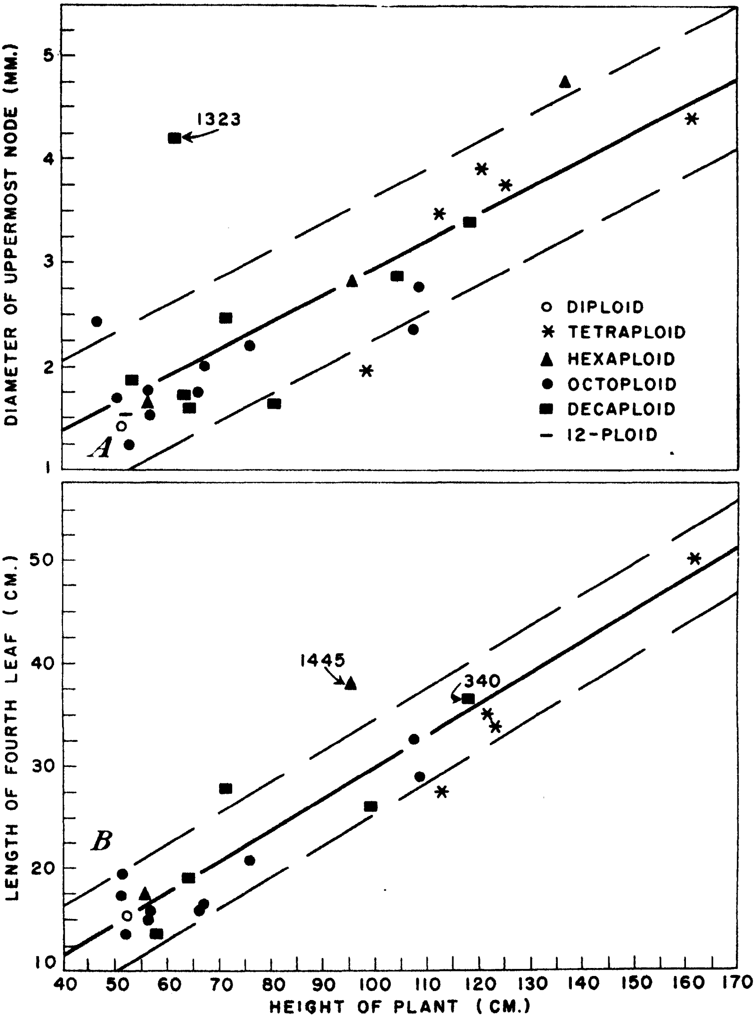


FIGURE 4.—Calculated regression and standard error of estimate for (*A*) diameter of uppermost node and (*B*) length of fourth leaf to height of plant. Actual magnitudes of these characters plotted under symbols shown.

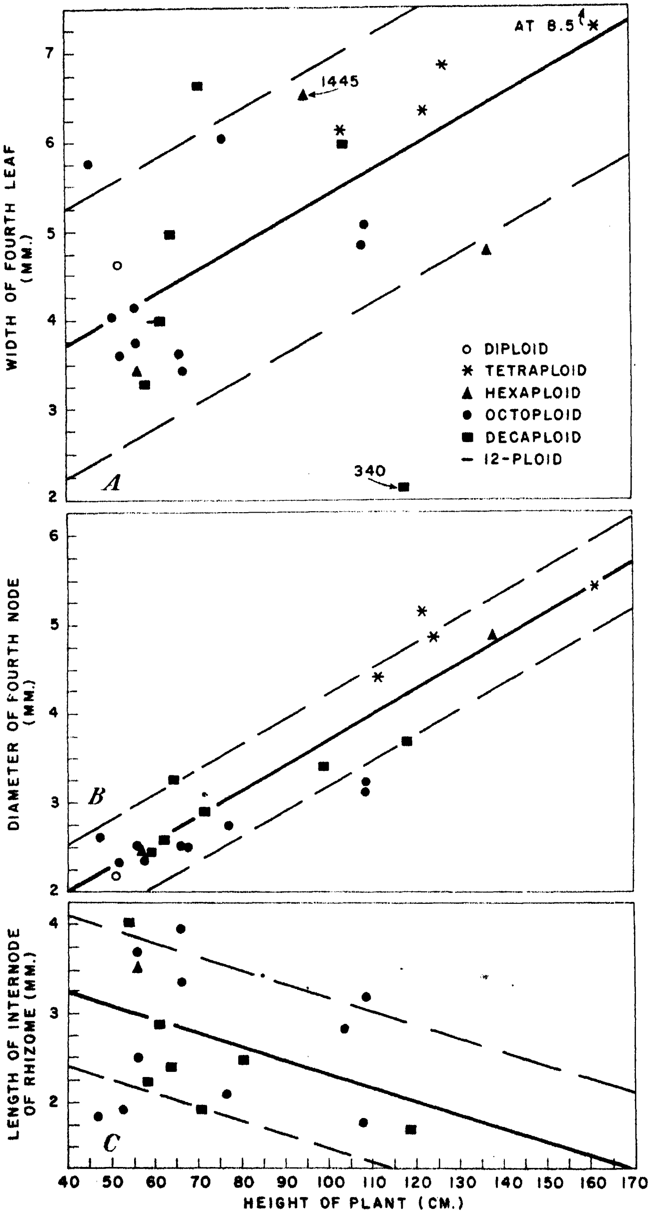


FIGURE 5.—Calculated regression and standard error of estimate for (*A*) width of fourth leaf, (*B*) diameter of fourth node, and (*C*) length of internode of rhizome to height of plant. Actual magnitudes of these characters plotted under symbols shown.

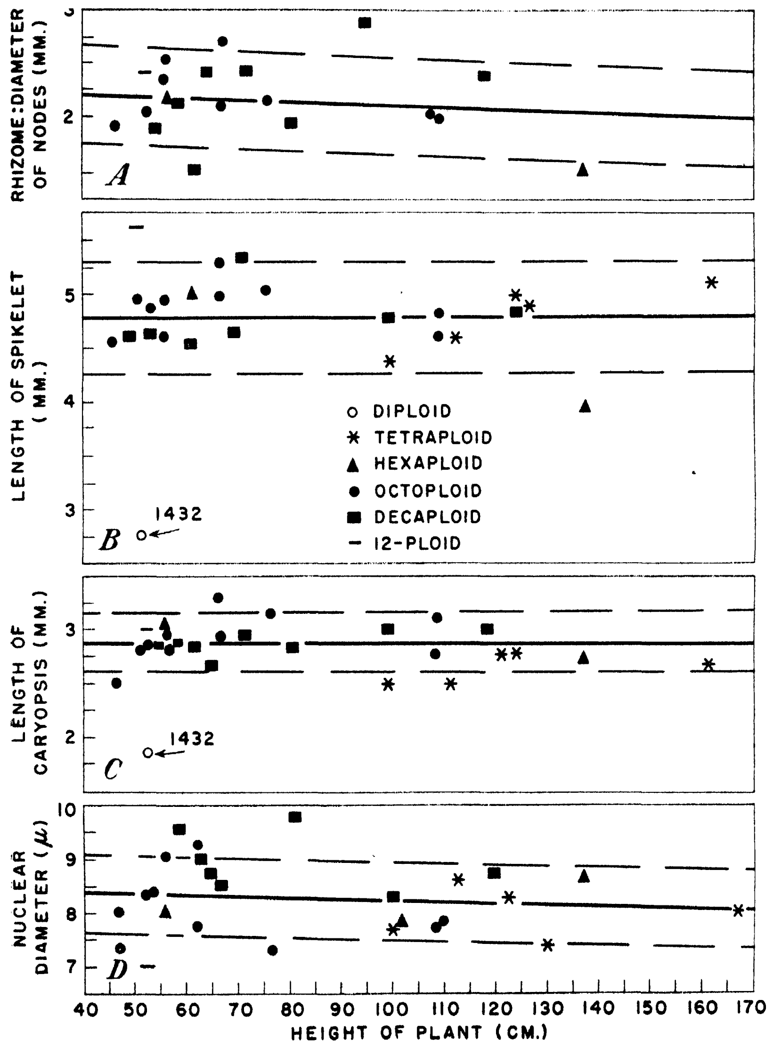


FIGURE 6.—Calculated regression and standard error of estimate for various characters to height of plant. Actual magnitudes of these characters plotted under symbols shown. *A*, Diameter of node of rhizome; *B*, length of spikelet; C, length of caryopsis; *D*, nuclear diameter.

the total number of comparisons made and the percentage of these comparisons that differ at the 1-percent level of significance. The comparison between isolates of different chromosome complexes (table 3) shows that when the "A" isolates are considered for all characters, 66.4 percent of the 7,328 comparisons differed significantly. For the "W" isolates 61.7 percent of the 412 comparisons differed significantly.

Table 3 shows also that 58.6 percent of the total number of "A" comparisons possible between characters of isolates of the same chromosome number differed by a significant amount. Twenty-nine, or 53.7 percent, of the 54 "W" comparisons made were between characters that differed at the 1-percent level of significance.

The spread in the percentage of differences regarded as significant is about 5 percent within complex and between complex pairing. It seems possible that this difference might have been introduced, in part at least, by moving isolates into a locality that differed edaphically, climatically, or topographically from that in which they originally grew. Such a population has in this analysis shown that the comparisons between characters of "A" isolates gave about 5 percent more significant t values than did the comparisons between characters of "W" isolates of the same species, taken from a situation to which those lines are considered naturally adapted.

The data presented for the isolates compared seem to indicate that in a population of plants, of this species at least, more than one-half of the isolates selected differ significantly from one another.

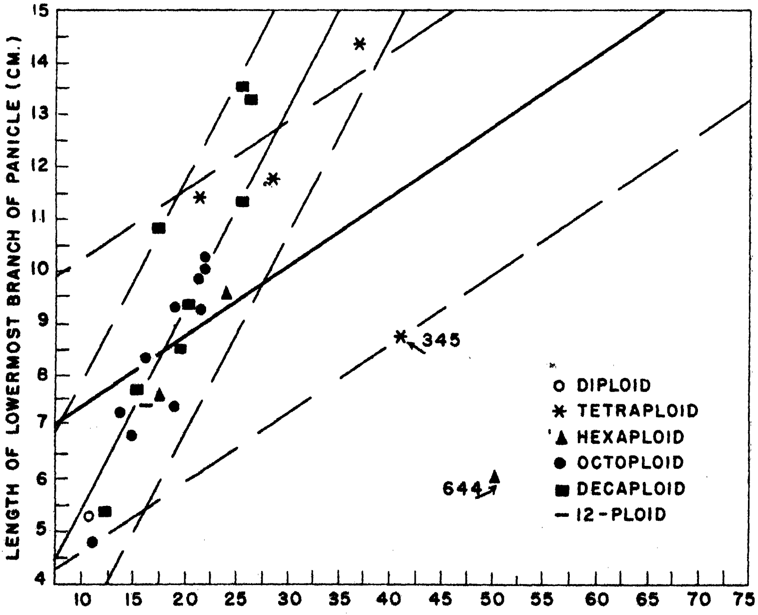


FIGURE 7.—Calculated regression and standard error of estimate for length of lowermost branch of panicle to length of panicle. Actual magnitudes of these characters plotted under symbols shown.

TABLE 3.—*Summary of all comparisons made* [1]

Comparisons	Isolates in population			
	A		W	
	N [2]	Percent [3]	*N* [2]	Percent [3]
BETWEEN ISOLATES OF DIFFERENT CHROMOSOME NUMBERS				
Diploids with all others	333	73.3	72	76.4
Tetraploids with all others	1,363	74.4	60	63.3
Hexaploids with all others	910	67.4	0	0
Octoploids with all others	2,354	63.6	130	58.5
Decaploids with all others	2,063	63.6	150	56.7
12-ploids with all others	305	60.7	0	0
Total	7,328	66.4	412	61.7
BETWEEN ISOLATES OF THE SAME CHROMOSOME NUMBER				
Tetraploid with tetraploid	118	66.1	0	0
Hexaploid with hexaploid	33	78.8	0	0
Octoploid with octoploid	648	51.1	15	26.7
Decaploid with decaploid	376	67.6	39	64.1
Total	1,175	58.6	54	53.7

[1] 15 characters considered.
[2] *N*=number of comparisons possible.
[3] Percent=percentage of comparisons significantly different at the 1-percent level.

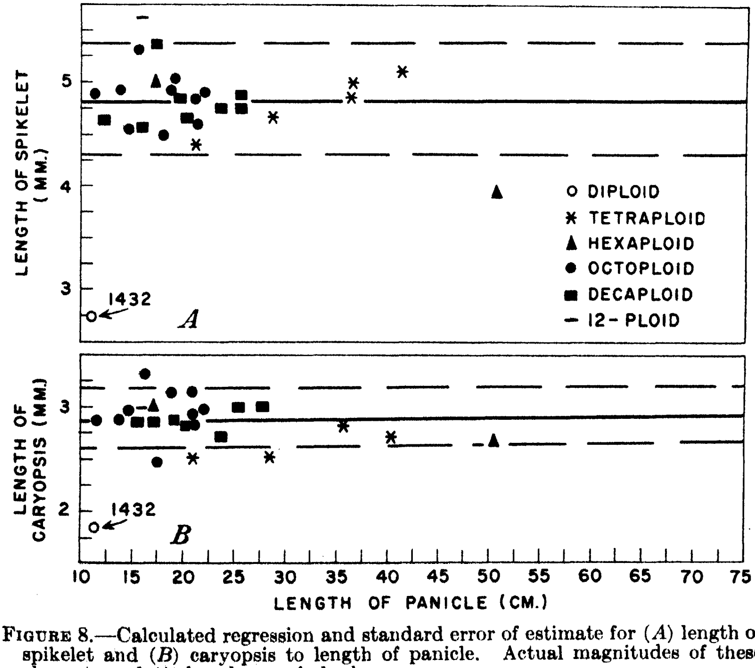


FIGURE 8.—Calculated regression and standard error of estimate for (*A*) length of spikelet and (*B*) caryopsis to length of panicle. Actual magnitudes of these characters plotted under symbols shown.

REGRESSION

In order to ascertain and analyze the variation of several characters with respect to one another, the data were studied by the analysis of variance applied to regression. The regression lines and lines above and below these at a distance of the standard error of estimate are given in figures 2 to 8, inclusive. Of these, figures 2 to 6, inclusive, show the regression of height of plant with the other characters considered. Figures 7 and 8 show the regression of the length of the panicle with the length of the lowermost branch of the panicle, the length of the spikelets, and the length of the caryopsis. The data from which these regressions were plotted are given in table 4, which shows also the *F* values for the regression variance, the regression coefficients, the correlation coefficients, and the coefficients of determination. The means of the characters considered against the height of plant (figs. 2–6) or the length of the panicle (figs. 7 and 8) are plotted on the same charts with the calculated regression lines. In order to ascertain whether there was any clustering of the means within a chromosome number, each isolate of a particular chromosome complex was plotted under a symbol assigned to all isolates of that complex.

TABLE 4.—*Covariance of plant height and of panicle length with measurement of various organs*

COVARIANCE OF PLANT HEIGHT WITH OTHER CHARACTERS

Character	*N*	Variance of errors of estimate	*F* value (variance due to regression)	Regression coefficient	Correlation coefficient	Coefficient of determination
Length of panicle	28	21.40	82.17**	0.256	0.872**	75.96
Length of lowermost branch of panicle	28	5.67	9.15**	.044	.510**	26.03
Length of uppermost internode	28	45.94	7.72**	.115	.479**	22.90
Length of uppermost leaf	27	14.48	69.38**	.206	.857**	73.50
Width of uppermost leaf	28	.83	32.13**	.031	.743**	55.27
Diameter of uppermost node	28	.36	48.45**	.026	.807**	65.08
Length of fourth leaf	22	12.18	149.02**	.291	.939**	88.17
Width of fourth leaf	24	1.65	8.99**	.024	.539**	29.03
Diameter of fourth node	23	.17	109.51**	.028	.916**	83.19
Length of internodes of rhizomes	20	.78	3.50	−.015	−.404	16.29
Diameter of nodes of rhizomes	20	.13		−.001	−.093	.86
Length of spikelet	27	.27		.001	.015	.02
Length of caryopsis	27	.08		.0001	.009	.01
Nuclear diameter	28	.55		−.001	−.062	.38
COVARIANCE OF PANICLE LENGTH WITH OTHER CHARACTERS						
Length of lowermost branch of inflorescence	28	6.16	6.38*	0.130	0.444*	19.69
Length of lowermost branch of inflorescence [1]	26	1.25	127.16**	.389	.917**	84.12
Length of spikelet	27	.27		−.001	−.016	.03
Length of caryopsis	27	.08		−.001	−.020	.04

** = odds at least 99 : 1 against such differences being due to random chance; * = odds at least 19 : 1 against such differences being due to random chance.

[1] Isolates 345 and 644 removed.

It is noted that if the means of the characters considered are plotted against height of plant or length of panicle or both there is no striking tendency for isolates of a particular chromosome number to occur together. Conversely, the plotted points for the means of characters of isolates of any particular chromosome complex may be scattered along the entire calculated regression for that character.

Theoretically, for any one of the isolates of the population examined to be significantly different from other isolates in regard to a particular character, the plotted point for that isolate should lie outside at least twice the standard error of estimate from the regression of the whole population. Several isolates differ significantly in this respect and will be considered briefly.

Only one isolate, No. 644, a hexaploid from Rogers, Ark., fell outside of the expected range in the height of plant to length of panicle regression (fig. 2, *A*). This isolate has long panicles, which make up over a third of the height of the plant, and short lowermost branches of the panicle (figs. 2, *B*; 7).

Isolate 340, a decaploid from Greeley, Colo., has long uppermost internodes. The mean length of this character plotted against height of plant lies outside twice the standard error of estimate from the regression (fig. 2, *C*).

Isolate 1323, another decaploid from Greeley, has uppermost nodes that are larger than the expected. Its nodes have a mean diameter of 4.1 mm., whereas the expected diameter of the uppermost culm node for a plant of its height (61.6 cm.) is about 1.8 mm. (fig. 4, *A*).

Isolate 1445, a hexaploid of unknown origin, has fourth leaves somewhat longer than the expected. Their width is, however, within the expected range (figs. 4, *B*; 5, *A*). Contrasting sharply with this relationship is that of isolate 340, which has very narrow fourth leaves (2.1 mm.) whose lengths (36.9 cm.) are near the expected for plants 118.0 cm. high.

The short spikelets and caryopses of the diploid isolate 1432 were both found to lie outside the expected range based upon height of plant (fig. 6, *B* and *C*).

No isolate examined varied from the expected in regard to length or width of uppermost leaves, fourth-node diameter, internode length, rhizome-node diameter, or nuclear diameter (figs. 3; 5, *B* and *C*; 6, *A* and *D*).

Two interesting cases were noted in considering the relation between the length of the lowermost branch of the panicle and the length of the panicle. Isolate 644, already mentioned as having very long panicles relative to height of plant, and isolate 345, a tetraploid from Stillwater, Okla., have very short lowermost panicle branches. If these two isolates are considered as a part of the total population, the calculated regression does not approach the course of the plotted means for the relation of length of panicle to length of lowermost branch of panicle (fig. 7). If, however, these two cases are not included in the total population used for the calculation of the regression, the expected or calculated regression follows the plotted relationship rather closely.

A study of the relation between the length of the panicle and the length of the spikelets and caryopses shows that both of the last-named characters of isolate 1432 were shorter than the expected (fig. 8).

It has been shown by these regressions that (1) there are positive and highly significant regressions between the height of the plant and the magnitude (width, length, or diameter) of the other aerial vegetative characters, (2) the rhizomes are so variable that it is difficult to make any generalization concerning the diameter of their nodes or the length of their internodes, and (3) no relation was found between the height of plant and the length of either spikelets or caryopses.

The relations of the length of panicle to the length of the lowermost branch of the panicle, the length of spikelets, and the length of the caryopses are similar to those between height of plant and aerial vegetative characters or reproductive organs.

These data are in agreement with long-recognized relationships in grasses. Generally one may expect to find that the size of aerial vegetative organs increases in a concomitant manner. Certain exceptions exist, such as those indicated in the preceding data. That there was no relation between such morphological characters as height of plant and length of spikelets and of caryopses was also shown.

NORMAL DISTRIBUTION

In the preceding sections of this paper the chromosome numbers found in several isolates of *Panicum virgatum*, the morphological variability, and the concomitant variability that occurs between certain characters of these isolates were considered separately. In this section, an integration of the results previously indicated will be made. An outline of the plan follows:

(1) The variation between the smallest and the largest means of each of the characters of the isolates examined was considered as 100 percent. The smallest mean value for any particular character in the material examined was considered as at the zero point. The greatest mean value for this particular character would then be at the 100-percent point, and isolates having mean values intermediate between these two would consequently fall along the base line between the zero and the 100-percent point. By this procedure it is possible for a portion of a normal frequency distribution to lie beyond the zero and 100-percent points.

(2) Four isolates, Nos. 345, 1321, 1413, and 340, from the tetraploid, hexaploid, octoploid, and decaploid complexes, respectively, were selected from the total population. The diploid and 12-ploid isolates were not included because of small populations. The following factors were considered in the selection of the isolates: (*a*) The population used to establish the mean height must be sufficiently large to be reasonably accurate for that particular isolate; (*b*) the heights should be reasonably well distributed along the base line for this particular character. For other characters it is then possible to ascertain lateral movement along the base line with relative ease by referring back to normal frequency distribution curves of plant height.

(3) Normal frequency distribution for the magnitude of each of the 15 characters considered was calculated from the frequency distribution of the sample and plotted on the base line in their positions relative to the zero point. To determine these distribution curves, the standard deviations of the means were used; that is, if another sample was taken from the population of a particular isolate and the observations represented by a normal frequency curve, the chances would be 2 to 1 that the mean of that sample would fall within the standard deviation of the curve representing that particular line as presented in this paper. These curves are based upon the standard deviation of the mean rather than upon the standard deviation of the individual because the t values were based upon the standard error of the mean. This type of presentation permits the magnitude of the t value to be shown on the charts by the distance between a given pair of curves for two different races. The following formula was used (*21, pp. 123–150*) for the determination of the normal frequency distribution curves:

$$Y=\frac{N}{\sigma_n\sqrt{2\pi}}\cdot\frac{-x^2}{e^2(\sigma_n)^2}$$

Here Y is the distance along the ordinate axis beyond the mean; x is the distance along the abscissa beyond the mean; N is the total number of variates; σ_n is the standard error of the mean; e is log. 10^e (0.4343). The points thus derived were plotted to scale, making it possible not only to ascertain and show graphically the relative significance between two curves but also to give a concept of the relative dispersion of the populations considered.

It was assumed that if there was a regular and definite relation between the characters considered one could expect the normal distribution curve of a certain isolate to retain essentially the same relative position between the extremes (0 percent and 100 percent) of the several characters considered. There is no positive evidence that this is true. Inspection of figure 9, *A*, shows, for example, that isolate 1413 was the shortest isolate considered. In length of panicle it is 16 percent of the range above the zero point, or somewhat longer than the isolate with shortest panicles (fig. 9, *B*). The normal distribution of this character of isolate 1321 lies entirely within the limits of the distribution of isolate 1413. A consideration of the length of the lower-

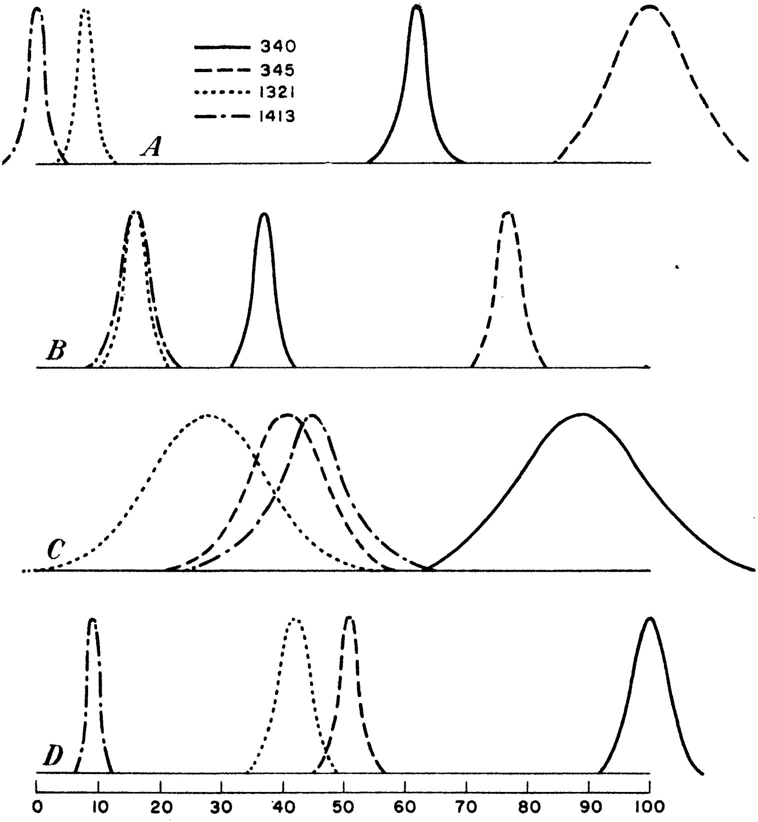


FIGURE 9.—Calculated normal frequency distribution curves for various characters of a tetraploid (345), a hexaploid (1321), an octoploid (1413), and a decaploid (340) isolate. The base line represents 100 percent of the mean range found in any particular character. The frequency distribution curves are arranged according to their sizes relative to the zero point, the mean of least magnitude for each character. The actual range in a given character is given with the character legend. *A*, Plant height (range 46.3–162.2 cm.); *B*, length of panicle (range 11.1–50.5 cm.); *C*, length of lowermost panicle branch (range 4.9–14.5 cm.); *D*, length of uppermost internode (range 5.7–39.4 cm.).

most branch of the panicle (fig. 9, *C*) shows that isolate 1413 has shifted positions in relation to the zero point and, for this character, lies somewhat above isolates 1321 and 345. That other isolates also vary in relation to those with which they are compared may be seen by following isolate 345 through the graphs (figs. 9–12).

The shape of the normal distribution curves indicates the relative dispersion of the characters of the isolates compared (figs. 9–12). As an example of this, one may consider the height-of-plant curves (fig. 9, *A*), which show the relative dispersions about the means for the four isolates. Isolate 345 has a rather broad dispersion as compared with those of other isolates shown. Other striking examples are the dis-

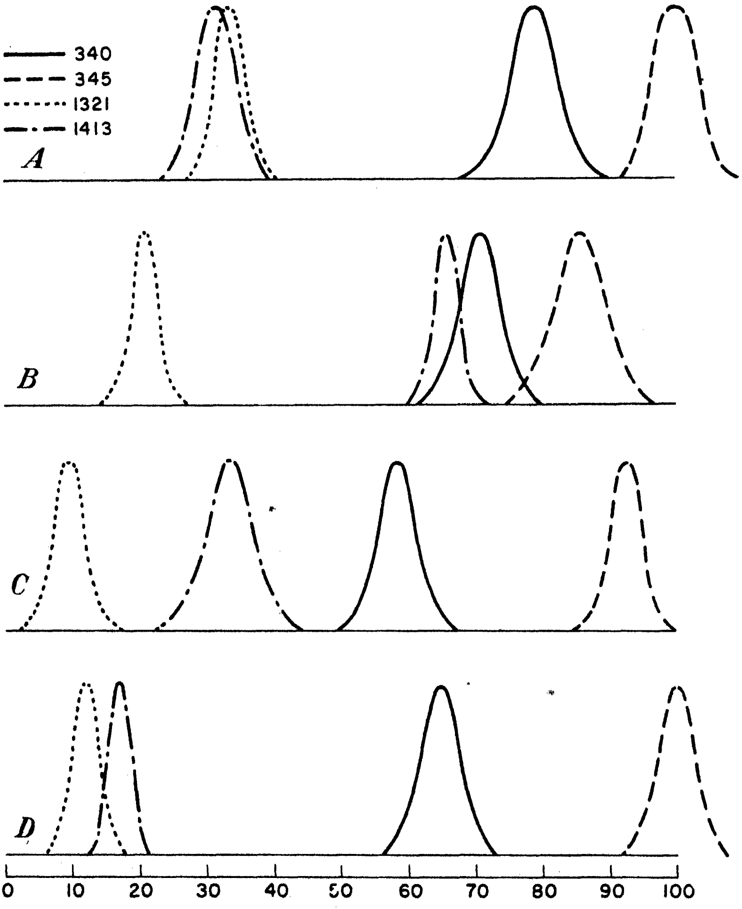


FIGURE 10.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Length of uppermost leaves (range 11.4–40.7 cm.); *B*, width of uppermost leaves (range 2.6–7.1 mm.); *C*, diameter of uppermost nodes (range 1.3–4.7 mm.); *D*, length of fourth leaves (range 12.8–49.8 cm.).

tribution curves for the reproductive organs (fig. 12, *A* and *B*). In the distribution curves for the length of the spikelets, there is but little difference in the dispersion of the four isolates considered. However, the case presented by the length of the caryopses is different. For this character the normal distribution of isolate 1321 lies entirely within the distribution curve of isolate 340. The configuration of the curves for isolates 340, 345, and 1321 are essentially similar and of relatively the same degree of dispersion. However, the distribution of the population of isolate 1413 is markedly different.

DISCUSSION

The data that have been discussed do not appear to aline themselves definitely with some of the observations reported for other species or genera. Investigations by Hagerup (*15*, *16*) and Tischler

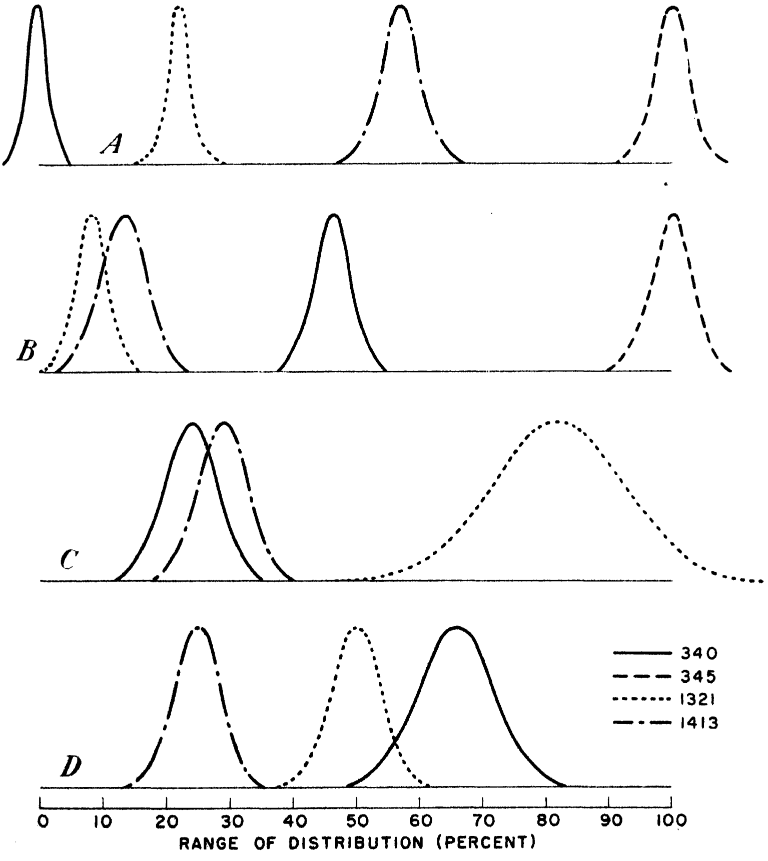


FIGURE 11.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Width of fourth leaves (range 2.1–8.5 mm.); *B*, diameter of fourth nodes (range 2.2–5.4 mm.); *C*, length of internodes of rhizomes (range 0.9–4.1 mm.); *D*, diameter of nodes of rhizomes (range 1.5–2.9 mm.).

(*31*) indicated that the percentage of polyploids is higher in climatically unfavorable regions than in regions where the climate is favorable. Hagerup (*17*) stated that * * * "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid form will not thrive." Müntzing (*22*, *p. 291*), in discussing autopolyploids, stated:

> Table 1 and the instances given above, however, suffice to demonstrate with absolute certainty that *chromosome races are ecologically different.* Only in one case (No. 18) is the possibility open that the races have the same ecological properties * * *. In fact, *not a single case is known in which it has been demonstrated that intraspecific chromosome races are ecologically identical.* In view of the data reported in table 1, it does not either seem likely that such cases will ever be found.

Certain discrepancies occur between these statements and the observations reported in this paper. The diploid isolate of *Panicum virgatum* was collected from west-central Wisconsin, where the climate must be considered continental. Other tetraploid isolates were found in an area extending from Wisconsin and North Dakota to Oklahoma and Texas. Church (*7*) reported tetraploid isolates of this species from Oklahoma and Massachussetts. Additional evidence that this species does not fall into natural chromosome complex ranges is found in other material collected from near Chippewa Falls, Wis. There material of all except the 12-ploid complex was collected from a small area. Likewise isolates of the hexaploid to 12-ploid numbers, inclusive, came from Greeley, Colo., another region

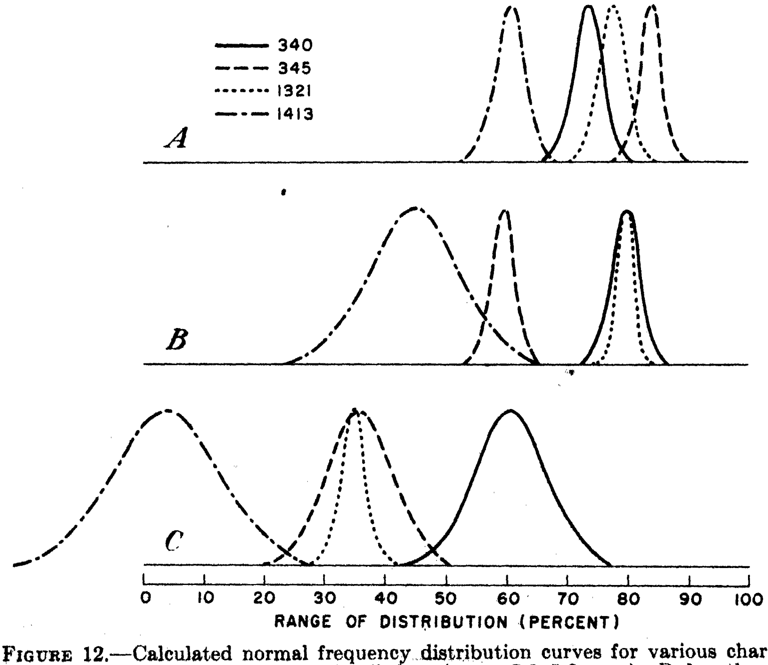


FIGURE 12.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Length of spikelets (range 2.8–5.6 mm.); *B*, length of caryopses (range 1.8–3.3 mm.); *C*, nuclear diameter (range 7.0μ–9.8 μ).

of continental climate. However, these observations support those of Clausen, Keck, and Hiesey (*8*) and Bowden (*3*) in that they show no definite regional segregation of chromosome number. Clausen et al. (*8, p. 423*) stated:

> * * * have emphasized that maritime plants tend to have high chromosome numbers. That this is no general rule is shown by the present investigations, where the coastal species are diploid in three complexes and tetraploid in only one. The investigations on chromosome number and distribution by the Tischler school are based on statistics in which a chromosome number is assigned to each species as a whole, not on counts of races from distinct habitats.

In a subsequent paragraph (*8, p. 424*) it is stated:

> Suggested correlations between chromosome numbers and size, environment, earliness, and other characteristics of plants have proved to be specific, not general.

The large number of different forms within the species *Panicum virgatum* presents a problem as to the taxonomic disposition of the segregates of grass species. There appear to be two methods that could be followed in classifying such segregates, and the success of either can be determined only through actual practice. It is not the purpose of this paper to attempt to outline in detail a definite system that might be workable. It is desirable, however, to suggest and to consider briefly the alternative schemes. The philosophy upon which these arguments are based holds that systematic botany should not be limited to the mere description of segregates but should also include an orderly and thorough analysis of characters and that the results of the investigations should be so presented that they will be of use not only to the specialist but to others as well.

The first of the alternative procedures here suggested as possible would be one whereby the different segregates would be described. This is, and long has been, the procedure generally followed by numerous systematists of both the Old World and the New. From the data presented it will be recognized that upon the basis of a statistical analysis such a procedure would be perfectly valid. It would entail the description of an almost innumerable number of segregates (species, subspecies, varieties, forms, etc.) the rank of which would be dependent to a large extent upon the judgment of the systematist. The importance individual taxonomists place upon different characters would enter as an additional complicating factor. Likewise, it should be recognized that if cytological differences are valid for taxonomic delimitation these differences would have to be, for the most part, ignored. There is little doubt that if such a procedure were followed it would not be long before the taxonomy of the Gramineae would assume the confused condition that now exists in *Crataegus* or *Rubus*. In some genera of grasses this status perhaps has been attained already.

An alternative procedure appears possible which incorporates certain features suggested in recent years by several European workers. This plan would necessitate the recognition of certain species of the Gramineae as being composed of a polyploid system made up of a considerable number of segregates within each one of the chromosome complexes. This concept of a species is essentially similar to Turesson's "coenospecies," but it may or may not be identical with it. Turesson (*32*) proposed the term "coenospecies" and defined it as including "the total sum of possible combinations in a genotype compound." The components of a coenospecies are related, so they may exchange genes among themselves to a limited extent through hybridization (*23*). The delineation of the component races of the polyploid system would not of necessity bear Latinized names but would be generally referred to only under the specific names of the species to which the races belong. The complexes of the polyploid system could be recognized by names such as *diplodium*, *tetraplodium*, *hexaplodium*, etc. (*12*). This nomenclature would readily indicate in more critical studies the general nature of the material under consideration. The validity of such a proposal is readily substantiated by reference to previously published papers (*1, 2, 4, 5, 6, 10, 11, 19, 20, 24, 27, 28, 29, 30*). These reports indicate that polyploid systems of chromosome numbers occur within species of *Bromus*, *Agropyron*, *Elymus*, *Paspalum*, *Andropogon*, and *Spartina*, to mention but a few of the genera wherein the aforementioned condition has been observed. Turesson (*33*) also proposed the term "ecospecies" as "the genotype compound narrowed down to the ecological combination limit." Clausen et al. (*8*) made the following statement in their glossary:

> *Ecospecies*, Turesson's term * * * used to denote a group of plants within the cenospecies whose members are able to interchange their genes without detriment to the offspring. Ecospecies are separated from one another by internal barriers that prevent such free interchange. They may or may not correspond to the Linnaean taxonomic species.

In *Panicum virgatum* this category would include a group of isolates of complexes of the coenospecies specialized to situation such as brackish marshes, open upland prairies, swales, etc. For such segregates of the ecospecies, the terms "ecotype" and "geo-ecotype," depending upon the ecological or geographical distribution, have been proposed (*32*). These isolates are essentially similar to those with which agronomists have been concerned. Gregor (*12*) proposed the term "agrotype" to designate the ultimate crop units that "owe their distinctive characteristics to conscious selection followed by controlled isolation." In the cultivated grasses such as oats, barley, wheat, etc., the broader classes under cultivation would be considered agro-ecotypes (*13, 14*).

Among the more common species of grasses there are commercial varieties or agrotypes, of varying homogeneity, of *Bromus inermis* Leyss. (Superior, Parkland), *Agropyron trachycaulum* (Link) Malte (Mecca, Grazier, Fyra), *Cynodon dactylon* (L.) Pers. (St. Lucie, Common, Giant, Tift), *Phleum pratense* L. (Huron, Shelby, Marietta), *Phalaris arundinacea* L. (Highland, Iowa 503), and *Agrostis palustris* Huds. (Metropolitan, Washington, Seaside, and Coos Bent). These few varieties and others not mentioned are rather distinct morphological types that do not, for the most part, bear Latinized names. The differences that separate them from other races of the same species are perhaps as distinctive as many that have been systematically recognized in species of wild populations. They suggest that frequently the similarity between the varieties of our cultivated species and native grasses has not been fully recognized. The multiplicity of forms of the cultivated grasses introduced from foreign countries in which they are native suggests that analyses of naturally occurring populations of these species would yield results that would essentially parallel those herein reported for *Panicum virgatum*.

SUMMARY

The chromosome numbers of 59 isolates of *Panicum virgatum* were determined. The seed and clonal stocks that were used for this study were taken from an area extending from Wisconsin and Montana south to Arkansas and Arizona. A polyploid series of 18, 36, 54, 72, 90, and 108 somatic chromosomes was determined. There does not appear to be any regional segregation of races of this species upon the basis of chromosome number.

Of the 59 isolates, 28 were compared statistically for the following quantitative characters: Height of the plant, length of the panicle, length of the lowermost branch of the panicle, length of the uppermost internode, length of the uppermost leaf, width of the uppermost leaf, diameter of the uppermost node, length of the fourth leaf, width of the fourth leaf, diameter of the fourth node, length of the internodes of the rhizomes, diameter of the nodes of the rhizomes, length of the spikelets, length of the caryopses, and the nuclear diameters in root-tip cells. In the 7,328 comparisons made between characters of all "A" isolates of different chromosome numbers, 66.4 percent of the comparisons indicated differences exceeding the 1-percent level of significance to occur between those characters. The 1,175 comparisons between all "A" characters of the same chromosome number indicated 58.6 percent of the characters differed to a highly significant degree. One diploid, one tetraploid, two octoploid, and three decaploid isolations obtained from Chippewa Falls, Wis., were considered separately in the same manner as the total population. Of the 412 comparisons made among Wisconsin "W" isolates of different chromosome numbers, 61.7 percent differed significantly. There were 54 comparisons between "W" isolates of the same chromosome number, of which 53.7 percent differed significantly.

The relations between height of plant and all other characters were studied by linear regression. It was ascertained (1) that there was a significant positive regression between the aerial vegetative organs and height of plant and (2) that there was no relation between height of plant and the lengths of spikelets and caryopses, the length of the internodes and diameters of the nodes of the rhizomes, or the diameters of the nuclei of root tips.

Regressions were calculated for the relation of the length of panicle to the length of the lowermost branch of the panicle, the length of the spikelets, and the length of the caryopses. A significant relation was found between the length of the panicle and its lowermost branch. The relations, however, between the length of the panicle and the length of the spikelets and caryopses were not significant.

Normal frequency distribution curves for each of the 15 characters of 4 isolates of different chromosome complexes were calculated and placed upon a common base line representing 100 percent of the variation found in that particular character. These curves indicated that wide differences separate certain lines for some characters, whereas for other characters such lines overlap or may be entirely inseparable.

Alternative procedures that could be followed in the systematic consideration of races of grass species are considered.

LITERATURE CITED

(1) AVDULOW, N. P.
1928. KARYO-SYSTEMATISCHE UNTERSUCHUNG DER FAMILIE GRAMINEEN. Allrus. Bot. Kong. Ber. 1928: 65–67.

(2) ———
1931. KARYO-SYSTEMATISCHE UNTERSUCHUNG DER FAMILIE GRAMINEEN. Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot., Genet., and Plant-Breeding) Suppl. 44, 428 pp., illus. [In Russian. German summary, pp. 353–425.]

(3) BOWDEN, W. M.
1940. DIPLOIDY, POLYPLOIDY, AND WINTER HARDINESS RELATIONSHIPS IN THE FLOWERING PLANTS. Amer. Jour. Bot. 27: 357–371, illus.

(4) BROWN, W. L.
1939. CHROMOSOME COMPLEMENTS OF FIVE SPECIES OF POA WITH AN ANALYSIS OF VARIATION IN POA PRATENSIS. Amer. Jour. Bot. 26: 717–723, illus.

(5) CHURCH, G. L.
1929. MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE. II. PANICEAE AND ANDROPOGONEAE. Bot. Gaz. 88: [63]–84, illus.

(6) ———
1936. CYTOLOGICAL STUDIES IN THE GRAMINEAE. Amer. Jour. Bot. 23: 12–15, illus.

(7) ———
1940. CYTOTAXONOMIC STUDIES IN THE GRAMINEAE SPARTINA, ANDROPOGON AND PANICUM. Amer. Jour. Bot. 27: 263–271, illus.

(8) CLAUSEN, J., KECK, D. D., and HIESEY, W. M.
1940. EXPERIMENTAL STUDIES ON THE NATURE OF SPECIES. I. EFFECT OF VARIED ENVIRONMENTS ON WESTERN NORTH AMERICAN PLANTS. Carnegie Inst. Wash., Pub. 520, 452 pp., illus.

(9) CORNELIUS, D. R., and JOHNSTON, C. O.
1941. DIFFERENCES IN PLANT TYPE AND REACTION TO RUST AMONG SEVERAL COLLECTIONS OF PANICUM VIRGATUM L. Amer. Soc. Agron. Jour. 33: 115–124, illus.

(10) FLOVIK, K.
1938. CYTOLOGICAL STUDIES OF ARCTIC GRASSES. Hereditas 24: [265]–376, illus.

(11) FULTS, J. L.
1942. SOMATIC CHROMOSOME COMPLEMENTS IN BOUTELOUA. Amer. Jour. Bot. 29: 45–55, illus.

(12) GREGOR, J. W.
1931. EXPERIMENTAL DELIMITATION OF SPECIES. New Phytol. 30: 204–217, illus.

(13) ———
1933. THE ECOTYPE CONCEPT IN RELATION TO THE REGISTRATION OF CROP PLANTS. Ann. Appl. Biol. 20: 205–219.

(14) ——— and HORNE, F. R.
1935. GROWTH FORMS: GENECOLOGY AND ITS AGRICULTURAL SIGNIFICANCE. Agr. Prog. 12: 89–98.

(15) HAGERUP, O.
1928. MORPHOLOGICAL AND CYTOLOGICAL STUDIES OF BICORNES. Dansk Bot. Arkiv 6: 1–[27], illus.

(16) ———
1932. ÜBER POLYPLOIDIE IN BEZIEHUNG ZU KLIMA, ÖKOLOGIE UND PHYLOGENIE. CHROMOSOMENZAHLEN AUS TIMBUKTU. Hereditas 16: [19]–40, illus.

(17) ———
1933. STUDIES ON POLYPLOID ECOTYPES IN VACCINIUM ULIGINOSUM L. Hereditas 18: [122]–128, illus.

(18) HITCHCOCK, A. S.
1935. MANUAL OF THE GRASSES OF THE UNITED STATES. U. S. Dept. Agr. Misc. Pub. 200, 1040 pp., illus.

(19) KARPER, R. E., and CHISHOLM, A. T.
1936. CHROMOSOME NUMBERS IN SORGHUM. Amer. Jour. Bot. 23: 369–374, illus.

(20) KATTERMANN, G.
1930. CHROMOSOMENUNTERSUCHUNGEN BEI GRAMINEEN. Planta 12: [19]-37.
(21) KELLEY, T. L.
1923. STATISTICAL METHOD. 390 pp., illus. New York.
(22) MÜNTZING, A.
1936. THE EVOLUTIONARY SIGNIFICANCE OF AUTOPOLYPLOIDY. Hereditas 21: [263]-378, illus.
(23) ——— TEDIN, O., and TURESSON, G.
1931. FIELD STUDIES AND EXPERIMENTAL METHODS IN TAXONOMY. Hereditas 15: 1-12.
(24) NIELSEN, E. L.
1939. GRASS STUDIES. III. ADDITIONAL SOMATIC CHROMOSOME COMPLEMENTS. Amer. Jour. Bot. 26: 366-372, illus.
(25) ——— and HUMPHREY, L. M.
1937. GRASS STUDIES. I. CHROMOSOME NUMBERS IN CERTAIN MEMBERS OF THE TRIBES FESTUCEAE, HORDEAE, AVENEAE, AGROSTIDEAE, CHLORIDEAE, PHALARIDEAE AND TRIPSACEAE. Amer. Jour. Bot. 24: 276-279, illus.
(26) SNEDECOR, G. W.
1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. 341 pp., illus. Ames, Iowa.
(27) SOKOLOVSKAYA, A. P.
1938. A CARYO-GEOGRAPHICAL STUDY OF THE GENUS AGROSTIS. Cytologia 8: 452-467, illus.
(28) STÄHLIN, A.
1929. MORPHOLOGISCHE UND ZYTOLOGISCHE UNTERSUCHUNGEN AN GRAMINEEN. II. UNTERSUCHUNGEN ÜBER DEN ZUSAMMENHANG VON ZYTOLOGIE UND MORPHOLOGIE, VON ZYTOLOGIE UND PHYLOGENIE BEI EINIGEN GRAMINEENGATTUNGEN. Wiss. Arch. f. Landw., Abt. A, Arch. f. Pflanzenbau 1: 342-398, illus.
(29) STEBBINS, G. L., JR., and LOVE, R. M.
1941. A CYTOLOGICAL STUDY OF CALIFORNIA FORAGE GRASSES. Amer. Jour. Bot. 28: 371-382, illus.
(30) STRELKOVA, O.
1938. POLYPLOIDY AND GEOGRAPHO-SYSTEMATIC GROUPS IN THE GENUS ALOPECURUS L. Cytologia 8: 468-480, illus.
(31) TISCHLER, G.
1935. DIE BEDEUTUNG DER POLYPLOIDIE FÜR DIE VERBREITUNG DER ANGIOSPERMEN . . . Bot. Jahrb. 67: 1-[36].
(32) TURESSON, G.
1922. THE SPECIES AND THE VARIETY AS ECOLOGICAL UNITS. Hereditas 3: [100]-113, illus.
(33) ———
1922. THE GENOTYPICAL RESPONSE OF THE PLANT SPECIES TO THE HABITAT. Hereditas 3: [211]-350, illus.

O

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., NOVEMBER 1, 1944 No. 9

SOME EFFECTS OF THE WAXY AND SUGARY GENES ON ENDOSPERM DEVELOPMENT IN MAIZE[1]

By R. H. ANDREW, *instructor in agronomy*, R. A. BRINK, *professor of genetics*, and N. P. NEAL, *professor of agronomy and genetics, Wisconsin Agricultural Experiment Station*[2]

INTRODUCTION

Two genetic factors in maize, waxy (*wx*) and sugary (*su*) and their respective dominant alleles, nonwaxy (*Wx*) and nonsugary or starchy (*Su*), so named because of their influence on storage products of the endosperm, are considered in this investigation. Attention is given to the effect of these genes on moisture content, carbohydrate content, weight, and morphology of the kernel; pericarp tenderness as measured by resistance to puncture; and canning quality.

Salisbury (*21*),[3] Atwater (*2*) and Richardson (*20*), working during the last century, demonstrated that mature kernels of sweet corn have approximately the following chemical composition: Carbohydrate, 65–75 percent; fat, 6–9 percent; protein, 10–12 percent. Appleman and Eaton (*1*) found a progressive decrease in total sugars, an increase in fat, and a very great increase in starch during development of the seed. Culpepper and Magoon (*7* and *8*), working with varieties of flint, floury, waxy, dent, and sweet corn, took samples of the growing ears at 5-day intervals from the 5th to the 30th day after pollination for chemical analyses. They stress that a distinctive feature of sweet corn is a high percentage of water-soluble polysaccharides consisting of dextrin and soluble starch. For instance, dent corn had 9.03 percent water-soluble polysaccharides on a dry-weight basis, 20 days after pollination, whereas sweet corn had 28.17 percent. The only other class of corn having a water-soluble polysaccharide content comparable to that of sweet corn was waxy, with 23.65 percent on the 20th day after pollination. However, this proportion fell sharply to 8.30 percent on the 25th day after pollination and remained low, as in dent and floury varieties. Similarly, Brink and Abegg (5), working on mature corn, found no differences in the percentage of water-soluble polysaccharides between waxy and nonwaxy varieties.

Waxy endosperm has been known in corn since 1909 when Collins (*6*) described the character in a new variety from China. Waxy seeds are opaque, light-colored, and if cut show a surface resembling that of wax. Weatherwax (*26*) noted the red staining reaction of waxy-maize starch with iodine and attributed the color reaction to the presence of erythrodextrin. Starchlike substances which give the red reaction with iodine have been found in other species of Gramineae. Meyer

---

[1] Received for publication April 19, 1943. Contribution from the Departments of Agronomy (Paper No. 184) and Genetics (Paper No. 317), Wisconsin Agricultural Experiment Station.

[2] The writers gratefully acknowledge their indebtedness to the late Dr. W. E. Tottingham, Department of Biochemistry, for suggestions with regard to the chemical analyses and Dr. J. H. Torrie, Department of Agronomy, for help with the statistical analysis.

[3] Italic numbers in parentheses refer to Literature Cited, p. 370.

(*15*) reported that the endosperms of certain rices and sorghums contained some starch granules straining blue, some amylodextrin staining red, and some dextrin staining brown. Harrison (*12*), studying the color reactions between starch and iodine, attributed the red and blue colors to differences in degree of dispersion of the iodine particles. Brink (*4*), using malt amylase as a reagent, reported that waxy and nonwaxy starch are alike with respect to the ultimate sugar unit of which they are composed, namely, maltose. He suggested that the difference between the two starches may be one of degree of association of a common fundamental molecule.

Lampe (*14*) made a microchemical and morphological study of the developing endosperm of maize. She found that presence of the dominant factor (*Su*) in the endosperm cells results in development of rather large, simple grains of carbohydrate, whereas in sweet corn (*su*) compound grains of carbohydrate and globules of liquid dextrin are formed.

Mechanical tests have been used by a number of workers in studies upon maturity of fruits and vegetables, on the assumption that, with increasing maturity, alterations in the tissues occur which affect resistance to puncture. Accepting this idea, Culpepper and Magoon (*7* and *8*) devised a "puncture meter" for corn; this is now produced commercially. Kernels are tested while still on the cob by placing the needle of this instrument at their apex and applying pressure until the pericarp is penetrated Resistance of the kernel to puncture is indicated by the reading just before penetration, when a sharp reaction of the spring and indicator occurs. Culpepper and Magoon found a rapid increase in toughness of corn kernels as development proceeds. They found also that the different classes of corn fall into two distinct groups with respect to toughness: Flour, flint, and waxy varieties become extremely tough as the season advances, whereas the sweet and dent varieties were more tender at corresponding ages.

Doxtator (*9*), studying quality in canning corn by use of the puncture meter, found that inbreds with low puncture pressures gave relatively tender hybrids when crossed with each other. He did not find a significant difference in pericarp thickness among varieties. On the other hand, Bailey and Bailey (*3*) report that varieties with the lowest puncture index at a given stage have the thinnest pericarps. Haddad (*10*) has reported on the morphological development of the pericarp in two inbred lines of sweet corn and their F_1 hybrid. He found that the pericarp decreases in thickness and becomes more resistant to puncture as maturity advances.

EXPERIMENTAL MATERIALS AND METHODS

In order to obtain material for the various comparative studies, waxy sweet corn (*suwx*) was pollinated with 4 types of pollen: Nonsugary, nonwaxy (*SuWx*); sugary, nonwaxy (*suWx*); nonsugary, waxy (*Suwx*); and sufary, waxy (*suwx*). A waxy strain of the hybrid Golden Cross Bantam was used as the seed parent. Golden Cross Bantam is a single hybrid, produced by controlled pollination of two inbred lines of yellow sweet corn, Purdue 39 and Purdue 51, which were developed by Smith (*23*). The parent stocks for the waxy hybrid used in this work were developed by outcrossing to a waxy stock and making the appropriate backcrosses. By 1939 Purdue

39 (*Wxwx*) had been backcrossed 3 times and Purdue 51 (*Wxwx*), 4 times. Some waxy Purdue 51 seed (*wxwx*) was also available. In order to obtain waxy Golden Cross Bantam seed in 1939 for the 1940 studies, heterozygous Purdue 39 plants (*Wxwx*) in the third backcross generation were pollinated with the waxy strain of Purdue 51 (*wxwx*), also 3 generations backcrossed. In the fall of 1939, 41 ears were obtained, segregating 50 percent waxy (*wxwx*) and 50 percent nonwaxy (*Wxwx*)kernels. The waxy kernels, planted in the spring of 1940, gave rise to plants which theoretically possessed 93¾ percent of the germ plasm of the original, nonwaxy Golden Cross Bantam corn. The stock used for studies a year later theoretically possessed 96⅞ percent of the germ plasm of standard Golden Cross Bantam.

As mentioned earlier, plants grown from the waxy Golden Cross Bantam seed (*suwx*) in 1940 and 1941 were pollinated with four types of pollen which differed in the genes for the waxy and sugary characters. Pollen carrying the surgary and waxy genes (*suwx*) was obtained from the waxy selection of Golden Cross Bantam; *suWx* pollen, from a commercial strain of Golden Cross Bantam; *Suwx* pollen, from a waxy double hybrid of the dent type; and *SuWx*, from a strain of field corn. Table 1 shows the genotypes of the pollen applied, of the polar nuclei of waxy Golden Cross Bantam, and of the endosperms obtained for analytical work, as well as the phenotypes of these endosperms.

TABLE 1.—*Genetic character of the material used for endosperm studies*

Pollen applied to waxy Golden Cross Bantam	Polar nuclei of seed parent	Genotype of resulting endosperm	Phenotype of endosperm
Su Wx	*suwx*	*Sususu Wxwxwx*	Nonsugary, nonwaxy
su Wx	*suwx*	*sususu Wxwxwx*	Sugar, nonwaxy.
Suwx	*suwx*	*Sususuwxwxwx*	Nonsugary, waxy.
suwx	*suwx*	*sususuwxwxwx*	Sugay, waxy.

Since it was desired to make comparative studies on kernels of the same age, all pollinations were made under controlled conditions on the same day, July 31 in 1940, and July 30, 1941. The number was over 2,000 in both years. In 1940 practically 100 percent fertilization was attained, but in 1941, as a result of excessively high temperature and rain on the day of pollination, the ears obtained were poorly filled.

Ear samples were collected between 4 and 6 p. m. at intervals from the tenth to the seventieth day after pollination. Up to the twentieth day after pollination in 1940, 15 ears for each endosperm type were picked on each harvest date, but after the twentieth day, when the ears had increased considerably in size, the number was reduced to 10 ears per type. In 1941, however, because of a poor seed set, 25 ears for each endosperm type were taken during sampling. Ears for canning tests were collected on a larger scale as a separate experiment in 1940.

The samples were taken to the laboratory and husked. Tests were made on resistance of the pericarp to puncture by use of the puncture meter. Needles of 3 diameters were available for the puncture meter. In 1940 the medium size (diameter =0.048 inch) was used, and in 1941 the small size (diameter =0.025 inch). The kernels were then cut from the cob with a scalpel. Samples were taken for moisture determination and a few kernels were preserved in formalin-acetic-alcohol for histological study. In 1940, 100 kernels from each of 5 ears for each endorsperm type were taken for moisture samples. Evaporation was carried out in a Proctor-Schwartz over at 80° C. for 48 hours. In 1941, 3 composite moisture samples for each kernel type were taken from 25 ears. The remaining kernels were dried for 24 hours at 80° C. under forced draft and stored in half-pint milk bottles for use in carbohydrate analyses.

Chemical determinations were made of reducing sugars, total sugars, and water-soluble polysaccharides in the 4 kernel types at 12 stages of development in 1940 and at 8 in 1941. The dried kernels were cleaned of chaff by compressed air, broken to small pieces in a drug mill, and ground in a ball mill to pass an 80-mesh screen. Extraction of 5-gm. samples in 80 percent alcohol brings the sugars into solution. To obtain polysaccharides soluble in cold water, the residue from this extraction was transferred to flasks and 200 cc. of distilled water added and thoroughly shaken. The milky liquid was allowed to stand for 12 hours at room temperature and was then filtered by suction through infusorial earth. Inversion of sucrose and water-soluble polysaccharides was accomplished by concentrated HCl (specific gravity =1.1878). All sugar determinations were made by Bertrand's volumetric permanganate method as outlined by Heinze and Murneek (*13*).

The ears remaining after sampling was completed in 1940 were used to ascertain comparative dry weights of the 4-kernel types. Two hundred ears of each type were harvested at the 72-day stage, classified according to the number of kernel rows per ear, and shelled. Approximately 70 percent of the ears had 12 rows of kernels; 15 percent, 10 rows; and 15 percent, 14 rows. For each of these 3 classes of ears within each of the 4 endosperm types, 1,000 kernels were weighted and the weights corrected to a dry basis.

EXPERIMENTAL RESULTS

CARBOHYDRATES

The different types of corn do not vary significantly in the quantities of reducing sugar which they contain or in the rate of change of this carbohydrate during development. Since the results obtained in 1941 closely approximate those obtained in 1940, only figures for the latter year are presented. The sugary kernels generally showed a higher content of reducing sugar than the nonsugary kernels (table 2 and fig. 1). On the other hand the waxy gene did not have a uniform influence on the percentage of reducing sugars present. All four percentage curves dropped sharply up to the 16-day stage in 1940 and then leveled off under 3 percent. In 1941 the reducing sugar had already declined to below 5 percent by the 14-day stage, the first date of sampling. Prevailing high temperatures and clear days between time of pollination and first harvest unodubtedly contributed to this low amount of reducing sugar in 1941.

Total sugars were present in greatest quantity on the first harvest date and decreased continuously thereafter in both 1940 and 1941. The rate of decrease was rapid until the 16-day stage and more gradual thereafter (table 2 and fig. 1). The sweet kernels maintained a

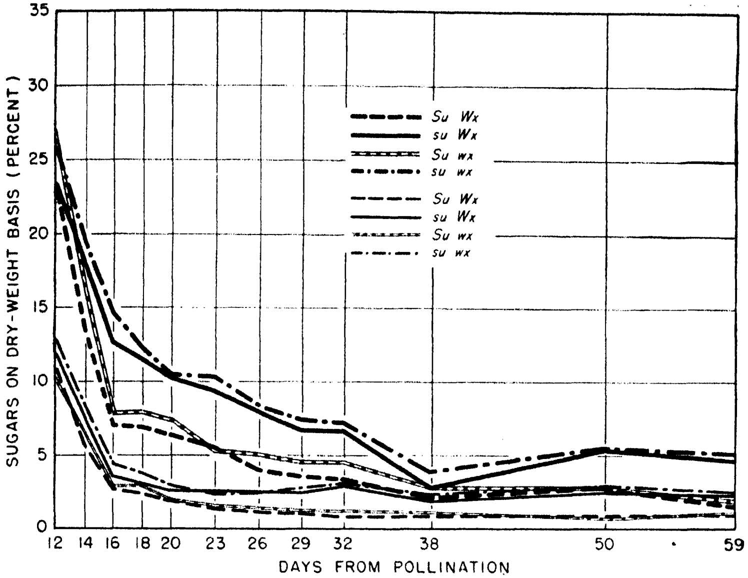


FIGURE 1.—Curves, showing the changes in sugar content for four kernel types during development in 1940 (heavy lines represent total sugars and light lines, reducing sugars).

TABLE 2.—*Chemical composition of 4 kernel types at 12 stages of maturity in 1940* [1]

Carbohydrate	Kernel type	Days after pollination											
		12	14	16	18	20	23	26	29	32	38	50	59
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Reducing sugar	*Su Wx*	10.81	5.72	2.60	2.43	1.96	1.45	1.13	1.08	.98	.84	.92	.83
	su Wx	11.93	7.52	3.54	3.06	2.61	2.60	2.54	2.51	2.86	1.97	2.51	2.36
	Suwx	10.11	6.58	2.95	2.93	1.98	1.67	1.41	1.25	1.23	1.06	.91	.84
	suwx	12.97	8.28	4.48	3.78	3.13	2.44	2.45	2.74	2.92	2.27	2.68	2.49
Total sugar	*Su Wx*	23.04	13.61	6.87	6.76	6.28	5.45	3.88	3.52	3.31	2.20	2.68	1.52
	su Wx	23.19	18.05	12.56	11.45	10.12	9.38	7.79	6.60	6.59	2.78	5.33	4.63
	Suwx	26.45	15.99	7.74	7.80	7.44	5.34	5.03	4.58	4.51	2.82	2.78	1.98
	suwx	26.18	19.47	14.61	12.19	10.45	10.28	8.26	7.34	7.17	3.87	5.38	5.01
Water-soluble polysaccharides	*Su Wx*	2.87	2.26	2.27	3.28	4.34	5.12	4.05	4.28	4.34	4.81	5.11	6.60
	su Wx	5.16	13.47	18.93	32.20	33.48	33.77	36.49	37.10	37.41	31.25	33.04	27.95
	Suwx	3.07	2.97	3.37	3.76	6.90	8.15	5.42	6.40	7.18	7.64	9.79	8.19
	suwx	3.94	15.49	23.55	35.11	37.42	37.61	38.72	40.66	42.08	34.43	40.72	36.28

[1] Carbohydrates calculated as glucose and expressed as percentage of dry weight.

greater percentage of total sugars throughout. Waxy kernels showed a slightly higher percentage of total sugars than nonwaxy in 35 out of 40 comparisons within the sugary and starchy classes in both years. However, this consistent difference possibly may be due to removal of some dextrins by the 80 percent alcohol extraction; more being removed from the waxy than from the nonwaxy type.

When the residues which remained after removal of the sugars were shaken in water and allowed to stand 12 hours, they gave supernatant liquids which were very difficult to filter. The solution from the nonsugary kernels was almost transparent while that from the sugary kernels was cloudy. No differences in appearance of the supernatant liquids were noted between waxy and nonwaxy types within the sugary and nonsugary classes.

Iodine tests were made on the water-soluble fraction for one harvest date in 1940, namely, at the 18-day stage. Four distinct tints resulted when a drop of very dilute IKI solution was used in making the test on a spot plate. If a drop of more concentrated IKI solution (0.3 percent potassium iodide and 1 percent iodine) is added four shades of red are obtained. A description of the color reactions is presented in table 3.

TABLE 3.—*Reaction of water-soluble fraction from 4 kernel types with iodine* potassium iodide

Kernel type	Color with dilute IKI	Color with concentrated IKI
Su Wx	Bluish purple	Brown-red.
su Wx	Grayish purple	Orange-red.
Suwx	Reddish	Purple-red.
uwx	Reddish yellow	Yellow-red.

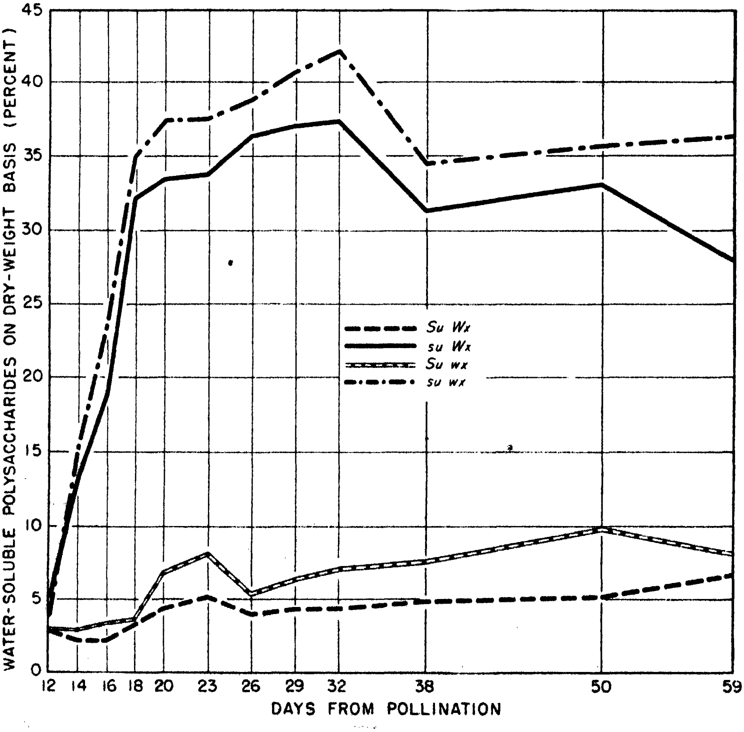


FIGURE 2.—Curves showing the changes in water-soluble polysaccharide content for four kernel types during development in 1940.

Purple tints obtained with dilute IKI for the nonwaxy endosperms indicate presence of some soluble starch in these varieties. The different shades of red with concentrated IKI indicate dextrinlike compounds in all four endosperms.

It is apparent from table 2 and figure 2 that the sugary gene is more influential than the waxy in determining the amount of the water-soluble fraction. In no case did the water-soluble fraction exceed 10 percent in the nonsugary kernels, whereas this percentage rose as high as 42.08 percent in the sugary types. The waxy gene, however, increases the water-soluble fraction in both the sugary and nonsugary classes.

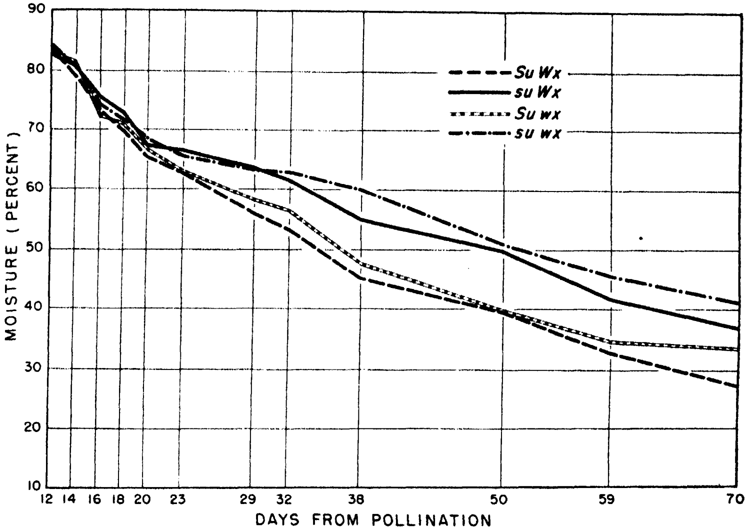


Figure 3.—Curves showing the changes in moisture content for four kernel types during development in 1940.

MOISTURE

Although the types of corn did not vary significantly in percentage of moisture at early stages of development, differences in the rates of moisture decline for the sugary and nonsugary classes became apparent after the 20-day stage (table 4 and fig. 3). The sweet types lose moisture less rapidly. Waxy kernels generally had a greater per-

Table 4.—*Moisture as percent in 4 kernel types at 15 stages of maturity in 1940*[1]

MOISTURE ANALYSIS

Kernel type	Days after pollination														
	5	8	10	12	14	16	18	20	23	29	32	38	50	59	70
Su Wx	90. 52	88. 34	87. 33	83. 66	79. 07	73. 50	69. 60	65. 80	62. 32	55. 93	53. 35	45. 11	39. 44	32. 78	26. 67
su Wx	90. 13	88. 37	87. 30	82. 96	80. 91	75. 20	72. 72	67. 53	66. 18	63. 78	61. 90	54. 96	49. 78	41. 72	36. 89
Suwx	90. 64	88. 89	87. 63	83. 10	81. 31	72. 66	71. 18	66. 41	63. 09	58. 26	56. 61	47. 87	39. 93	34. 77	33. 86
suwx	90. 36	88. 28	87. 57	84. 10	80. 94	73. 97	71. 60	68. 12	66. 07	63. 73	62. 97	60. 00	50. 52	45. 80	41. 06

---

[1] Minimum significant difference between means=2.21 percent.

Table 4.—*Moisture as percent in 4 kernel types at 15 stages of maturity in 1940*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	14	6, 604. 0	2, 096. 51**
Kernel types	3	479. 2	152. 13**
Interaction (harvests × types)	42	37. 6	11. 93**
Error	240	3. 2	
Total	299		

** = Significant at the 1-percent level.

centage of moisture than nonwaxy within the sugary and nonsugary classes, although all the differences are not statistically significant. The minimum significant difference in moisture content between any two types on any harvest date in 1940 is 2.21 percent.

It is of interest to note the length of time after pollination before the amount of dry matter in the different kernel types reaches 50 percent (table 5). The amount of dry matter reached 50 percent of the total weight at earlier stages of development in the starchy kernels than in the sugary. In this respect starchy types averaged 15 days ahead of sugary types in 1940 and 12 days ahead in 1941. Within the nonsugary and sugary classes the nonwaxy types were ahead of the waxy types. It is to be noted that in 1941 the time required for the dry matter to reach 50 percent averaged 10 days less for all kernel types than in 1940. The difference may be attributed both to the weather conditions and to the fact that the ears in 1941 were not well filled.

Table 5.—*Approximate time when dry matter reaches 50 percent in the 4 kernel types for the years 1940 and 1941*

Kernel type	Days from pollination in 1940		Days from pollination in 1941		Difference between years
	Non-sugary class	Sugary class	Non-sugary class	Sugary class	
					Days
Su Wx	34		24		10
su Wx		49		37	12
Suwx	36		29		7
suwx		51		41	10
Average	35	50	27	39	10

KERNEL WEIGHT

That more reserves are stored in nonsugary than in sugary endosperms is shown by the comparative dry weights of the different kernel types at maturity. Seventy-two days after pollination ears were classified on a basis of endosperm type and number of kernel rows, making 12 classes in all. Absolute dry weights were obtained for 1,000 kernels of each class. Table 6 shows the average dry weight in grams for 100 kernels of each class and figure 4 illustrates the results. The nonsugary types attain greater dry weights than the

TABLE 6.—*Dry weight of 100 kernels of 4 endosperm types from 3 classes of ears 72 days after pollination*

Rows of kernels on ears (number)	Endosperm type			
	Su Wx	*su Wx*	*Suwx*	*suwx*
	Grams	*Grams*	*Grams*	*Grams*
14	30.93	22.85	30.34	23.64
12	31.89	24.62	31.27	25.02
10	34.07	26.26	33.82	26.11

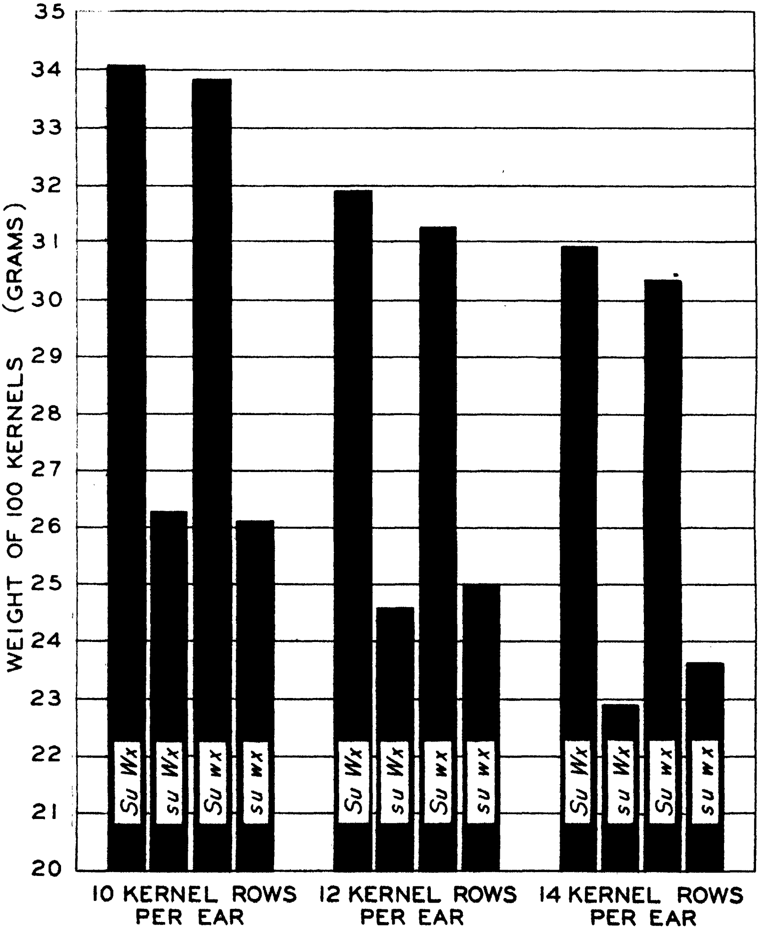


FIGURE 4.—Diagram showing graphically the dry weight of 100 seeds at the 72-day stage from ears with 10, 12, and 14 rows of kernels for each of 4 types of corn.

sugary types. For instance, *SuWx* kernels from 12-row ears had an average weight of 31.89 gm. per 100 seeds and *suWx* kernels, 24.62 gm. per 100.

TENDERNESS OF THE KERNEL

Data on resistance to puncture for the 4 kernel types which are presented in table 7 and in figure 5 represent the average values for 100 kernels (25 from each of 4 ears) in 1940, and for 120 kernels (15 from each of 8 ears) in 1941. Extensive discussion of the graphs is unnecessary, since it is obvious that the sugary and nonsugary endosperms fall into 2 distinct classes with respect to resistance to puncture. Nonsugary kernels were consistently tougher from the 12-day stage onward in 1940 and from the 14-day stage in 1941. At 26 days after pollination in 1940 and 31 days in 1941 it was impossible to measure a number of the nonsugary kernels because they offered more than 500-gm. resistance, the upper limit of the meter scale. There were no significant differences between the toughness of waxy and nonwaxy kernels within the sugary and nonsugary classes. Differences between any 2 kernel types for average resistance to puncture must be greater than 20 gm. in 1940, and in 1941, greater than 15 gm., in order to be statistically significant.

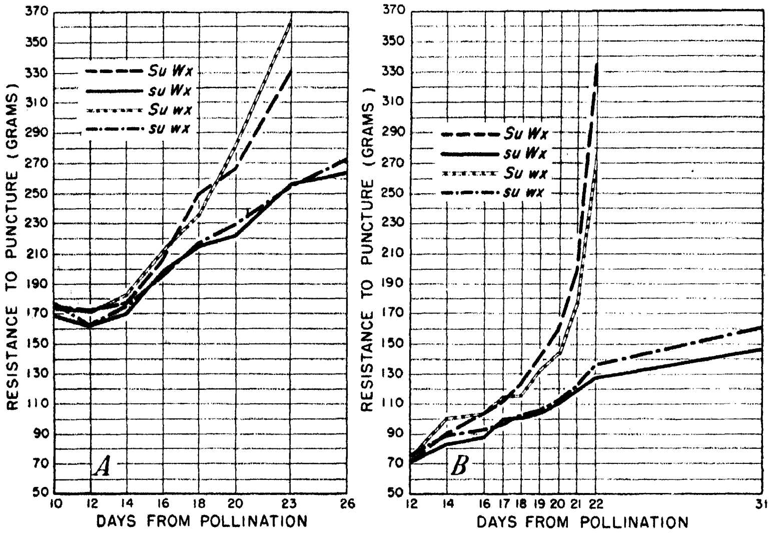


FIGURE 5.—Curves illustrating the increase in toughness of kernels for four types of corn in 1940 (*A*) and 1941 (*B*)

Since kernels with nonsugary endosperms proved to be much less tender than kernels with sugar endosperms, some related experiments were conducted in an attempt to evaluate the influence of the underlying tissue on the resistance which the pericarp offered to penetration. By 18 days after pollination the triploid endosperm tissue of maize occupies virtually all the space in the kernel except that taken by the embryo. The ovary wall has been transformed into a comparatively thin pericarp (*19*).

TABLE 7.—*Average pressure in grams required for pericarp penetration of 4 kernel types at 8 stages of maturity in 1940, and at 10 stages of maturity in 1941*

PRESSURE MEASUREMENTS, 1940[1]

Kernel type	Days after pollination							
	10	12	14	16	18	20	23	26
SuWx	175	172	177	207	251	268	333	
suWx	169	162	170	198	216	223	256	264
Suwx	173	171	182	211	236	283	364	
suwx	177	162	175	197	217	229	255	273

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	6	39,512.0	102.53**
Kernel types	3	7,719.5	37.61**
Interaction (harvests × types)	18	1,579.9	7.70**
Error	84	205.2	
Total	111		

PRESSURE MEASUREMENTS, 1941[2]

Kernel type	Days after pollination									
	12	14	16	17	18	19	20	21	22	31
SuWx	70	90	104	112	124	142	159	200	336	
suWx	71	83	88	99	101	105	111	120	129	148
Suwx	74	102	104	115	116	133	144	178	273	
suwx	76	89	93	97	103	106	112	122	136	161

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	*F* value
Harvests	8	59,487.1	262.95**
Kernel types	3	41,931.0	185.35**
Interaction (harvests × types)	24	8,193.5	36.22**
Error	252	226.2	
Total	287		

[1] Medium needle used (diameter = 0.048 inch). Minimum significant difference between means = 20 gm.
[2] Small needle used (diameter = 0.025 inch). Minimum significant difference between means = 15 gm.
** = Significant at the 1 percent level.

In one experiment two grades of paper, the so-called onionskin and 16-pound bond, respectively, were punctured over modeling clay of a heavy consistency and over window putty which was considerably more pliable. One hundred and fifty tests were made for each of the four combinations of paper and background. Table 8 shows the average values obtained in these tests. It is apparent that the underlying material, which corresponds to endosperm tissue in the corn kernel, has a profound effect on the puncture readings.

TABLE 8.—*Resistance to puncture offered by 2 types of paper over 2 types of background*

PUNCTURE MEASUREMENTS

Paper	Background	Average resistance
		Grams
Heavy	Modeling clay	444
Do	Putty	357
Onionskin	Modeling clay	223
Do	Putty	158

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variance or mean square	F value	Mean	Standard deviation	Coefficient of variation
				Grams		
Between treatments	3	1,711,982.75				
Within treatments:						
Heavy paper-clay background	99	967.54	1.92**	444	31.00	6.98
Heavy paper-putty background	99	1,062.96	2.11**	357	32.60	9.13
Onionskin paper-clay background	99	568.53	1.13	223	23.83	10.70
Onionskin paper-putty background	99	504.38	1.00	158	22.45	14.20
Total	399					

** = Significant at the 1-percent level.

A second experiment was designed to determine whether or not the sugary and starchy endosperm tissues had exerted a differential influence during development on the toughness of the hulls themselves. Pericarps from sugary, nonwaxy (*suWx*) and starchy, nonwaxy (*SuWx*) kernels at the 72-day stage were peeled and punctured. Peeling was accomplished by soaking the kernels for a short time in a dilute solution of sodium hydroxide, after which the tips were removed by a scalpel, and the hulls easily slipped off. Pieces of pericarp from the flat side of the kernel were laid over a washer held taut by a nut of small bore, and punctured. This simple test measured the resistance of the pericarp tissue alone, since there was no underlying support at the point of penetration.

Pericarps from sugary kernels offered an average resistance of 54 gm. for 250 tests, and those from nonsugary kernels, an average resistance of 75 gm. These differences are significant beyond the 1-percent point (table 9). Since the pericarps from both kernel types are alike

TABLE 9.—*Analysis of variance on resistance to puncture of peeled pericarps from sugary and nonsugary kernels*

Source of variation	Degrees of freedom	Variance or mean square	F value	Mean	Standard variation	Coefficient of variation
				Grams		
Between types	1	54,288.2	208.99**			
Within types:						
1. Su	249	265.6	1.09**	75	16.29	29.93
2. su	249	243.9		54	15.60	20.73
Total	499					

** = Significant at the 1-percent level.

genotypically, it must be assumed that they are influenced during development by the nature of the underlying endosperm tissue. The endosperm type is in turn determined by the type of pollen which is applied to the sugary, waxy parent (*suwx*). Swingle (*24*) has proposed the term "metaxenia" for the direct effect of the pollen on parts of the seed and fruit lying outside the embryo and endosperm. Waller (*25*), however, 11 years earlier, had proposed the term "ectogeny" for effects other than xenia, produced following fertilization. Such effects have been reported for the date palm (*18*), apple (*16 and 17*), cotton (*11*), and oak (*22*).

HISTOLOGICAL STUDIES

At the 16-day stage in 1940 the sugary and nonsugary kernels showed distinct differences in the nature and compactness of the storage products in the cells, but no differences were noted between waxy and nonwaxy kernels (fig. 6). The peripheral areas of all four endosperms, representing approximately one-third of the area in longitudinal section, were densely staining. In the nonsugary kernels, cells in this outer area were packed with simple starch grains (fig. 6, *A*). Toward the center of the kernel, however, there was a gradual decrease in the number of starch grains, until the cells in the basal region were without grains. On the other hand, the peripheral, storage region of the sugary kernels was composed of cells with two distinct types of reserves. Outer cells of this peripheral region were densely cytoplasmic, but showed no visible granules of solid carbohydrate

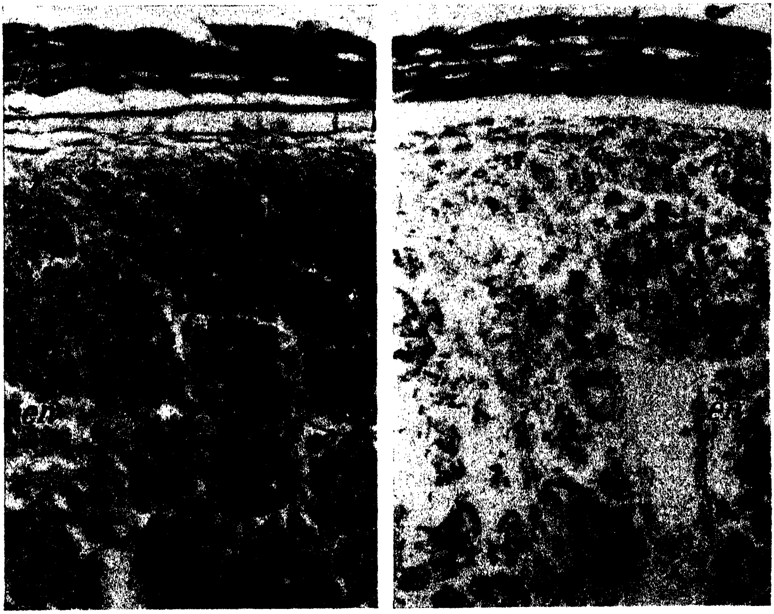


FIGURE 6.—Photomicrographs of longitudinal sections of corn kernels at the 16-day stage of development, showing the pericarp and the outer endosperm: *A*, *SuWx*; *B*, *suWx*; *p*, pericarp; *en*, endosperm. Ocular, × 10; objective, 16 mm.

(fig. 6, *B*). Inner cells of the peripheral region were densely cytoplasmic, but they also contained compound granules of solid carbohydrate. Although these carbohydrate granules were compound, they were no larger than the simple starch grains of the nonsugary kernels; nor were they as numerous as the grains in the nonsugary types. Progressively toward the center of the sugary kernels, cells were lighter staining and contained a decreasing number of granules, until the basal region was clear and without grains.

CANNING TRIALS

Canning trials in 1940 were conducted at 3 stages of maturity, 14, 16, and 18 days after pollination. Between 50 and 100 ears of each type were canned as a whole-kernel pack. Brine consisting of 14 pounds of salt and 40 pounds of sugar per 100 gallons of water was used. The cans of corn were given code numbers and graded by 4 persons.

Fourteen days after pollination the corn proved to be decidedly too young for satisfactory commercial canning. While the moisture content of the four endosperm types was approximately the same, the nonsugary, nonwaxy kernels (*SuWx*) were slightly mealy, even at this early stage. This characteristic may be attributed to the endosperm reserves. Only 2.26 percent water-soluble polysaccharides were present and starch grains were already filling the cells. The three remaining kernel types (*suWx*, *Suwx*, and *suwx*) were closely similar in taste and texture. That the nonsugary, waxy type (*Suwx*) should be graded in a class with the sweet corns, is rather unexpected, for it too had a low percentage of water-soluble polysaccharides, and the endosperm cells were filled with starch grains.

At the 16-day stage the sweet types were still a little young for good quality canned corn. Nonwaxy, sweet corn (*suWx*) had the best flavor on this date, but in general it was very similar to the waxy, sweet type (*suwx*), although the latter was slightly more tender and watery. The nonsugary, nonwaxy type (*SuWx*) was lacking in flavor, but was still judged more like the sweet corns than like the nonsugary, nonwaxy type. The relative compactness of the storage products of sweet and starchy corn, shown in figure 6, makes the differences in texture readily understandable.

Eighteen days after pollination, two lots of each type were picked. One of these lots was allowed to stand outside the factory in bags for 24 hours and the other was canned immediately. Of the corn which was canned immediately, the sweet types proved to be at or near their optimum canning stage. The waxy, sweet variety (*suwx*) was in an optimum condition and was judged best in quality; it was also considered slightly better in quality than the nonwaxy, sweet type (*suWx*) which had been harvested at the 16-day stage, although the two were quite similar. The results of these trials show that the waxy gene retards the development of sweet corn slightly, but they do not indicate that it produces a longer optimum canning period, for the waxy sweet corn was a little young at the 16-day stage, and the nonwaxy sweet was a little old at the 18-day stage; nonwaxy sweet corn could have been ideally canned at 16 or 17 days after pollination, and the waxy sweet, at 17 or 18 days. At 18 days, the nonsugary, nonwaxy type was very mealy and decidedly undesirable for eating purposes. The nonsugary, waxy type was strikingly different at this stage. It gave a thick, viscous product which resembled tapioca pudding in consistency and could not be poured from the can. It was relatively unpalatable.

After standing 24 hours, the two sweet corns gave canned products of a dull, flat flavor, indicating depletion of moisture and conversion of sugars and dextrins to starch. No differences in quality could be detected between the waxy and nonwaxy types.

DISCUSSION

Primarily the sugary and waxy genes influence the synthesis of polysaccharides in the endosperm cells of maize. Secondary effects are upon kernel weight, tenderness, and moisture content. Differences for all these qualities were greater between the sugary and nonsugary classes of kernels than between the waxy and nonwaxy classes.

Comment has already been made on the high percentage of water-soluble polysaccharides in sweet corn and the somewhat higher percentage of this fraction in the waxy, sweet kernels. It appears that introduction of the waxy gene into sweet corn varieties has promise as a means of increasing the water-soluble fraction which is so important in determining canning quality. On the basis of canning tests, waxy, sweet (*suwx*) was considered better than nonwaxy, sweet (*suWx*) when both types were harvested in optimum condition. On the other hand, while the delay in harvest, due to the waxy gene (*wx*), results in greater water-soluble polysaccharide content, it may also result in a tougher pericarp, since the waxy gene when homozygous delayed the optimum date of canning by a day.

It may be noted that a high percentage of sugar in the kernel is not of primary importance in determining canning quality. Sugars were present in maximum quantities for all types on the first dates of sampling, while the corn was not suitable for canning until several days later; differences between types were small.

The sugary gene when homozygous greatly reduces the resistance to puncture by modifying the nature and compactness of reserves in the endosperm cells. The differential influence of the *su* and *Su* genes on the resistance of the pericarp tissue itself, however, raises some interesting questions. Since the average dry weight attained by sugary kernels on 12-row ears was 24.62 gm. per 100, and by nonsugary kernels, 31.89 gm., it is apparent that absence of the nonsugary gene (*Su*) decreases the amount of total dry matter laid down in the endosperm. Do the effects of the *Su* and *su* genes in the endosperm also influence nutrition of the pericarp, since both the ovary wall and the endosperm are supplied by the same vascular tissue at the base of the kernel? Or does the difference in resistance have a mechanical explanation, the greater amount of reserves laid down in starchy endosperms resulting in greater stretching and increased growth of the pericarp?

The effects of the sugary and waxy genes on moisture depletion are interesting when it is recalled that the different kernel types developed on plants of the same genetic constitution. While it is undoubtedly true that maturity is controlled by many genes which influence such complex characters as growth rate, storage of food materials, and time of flowering, results of this work indicate that genes controlling endosperm type also influence the rate of maturation as measured by moisture content. Further and more accurate studies could be made if mixtures of sugary and nonsugary pollen were applied to the same ears and moisture samples taken as soon as the kernel types could be classified.

SUMMARY

Two pairs of alleles, waxy (*wx*) and nonwaxy (*Wx*), and sugary (*su*) and nonsugary (*Su*), which influence storage products of the endosperm of maize are considered in this work. To obtain material for analysis under similar environmental conditions, four types of pollen, *SuWx*, *suWx*, *Suwx*, and *suwx* were applied under controlled conditions to *suwx* plants. Samples were taken periodically during development for studies on carbohydrate content, moisture content, dry weight, pericarp tenderness, morphology of the kernel, and canning quality.

Chemical studies bear out earlier work showing that sugary endosperms contain a greater amount of water-soluble polysaccharides than nonsugary endosperms. Presence of the waxy gene in sweet corn further increases the percentage of the water-soluble fraction.

Effects of the sugary and waxy genes are not confined to synthesis of carbohydrate reserves in the endosperm. The sugary kernels lose their moisture less rapidly with advancing maturity, and within the sugary and nonsugary classes, waxy kernels generally show greater percentages of moisture than nonwaxy kernels. At maturity, nonsugary kernels have a greater dry weight than sugary kernels.

Different kernel types fall into two distinct classes with respect to resistance to puncture, the resistance of the starchy kernels increasing much more rapidly with greater maturity than that of sugary kernels. This difference is largely due to the nature and compactness of reserves in the endosperm cells. However, the pericarps themselves, which are genotypically alike, give differences in resistance when they are peeled and punctured, those from nonsugary kernels offering more resistance than those from sugary kernels. No significant differences in toughness were noted between waxy and nonwaxy kernels within the sugary and nonsugary classes.

Canning trials made at three stages of development indicate that neither of the starchy endosperms (*SuWx* and *Suwx*) give desirable products. The waxy gene delays the optimum canning stage of sweet corn for a day without, however, increasing the duration of the canning stage.

LITERATURE CITED

(1) Appleman, C. O., and Eaton, S. V.
1921. EVALUATION OF CLIMATIC TEMPERATURE EFFICIENCY FOR THE RIPENING PROCESSES IN SWEET-CORN. Jour. Agr. Res. 20: 795–805, illus.

(2) Atwater, W. O.
1869. ON THE PROXIMATE COMPOSITION OF SEVERAL VARIETIES OF AMERICAN MAIZE. Amer. Jour. Sci. (ser. 2) 48: 352–360.

(3) Bailey, D. M., and Bailey, R. M.
1938. THE RELATION OF THE PERICARP TO TENDERNESS IN SWEET CORN. Amer. Soc. Hort. Sci. Proc. 36: 555–559, illus.

(4) Brink, R. A.
1928. DYNAMICS OF THE WAXY GENE IN MAIZE. II. THE NATURE OF WAXY STARCH. Biochem. Jour. 22: 1349–1361, illus.

(5) ——— and Abegg, F. A.
1926. DYNAMICS OF THE WAXY GENE IN MAIZE. I. THE CARBOHYDRATE RESERVES IN ENDOSPERM AND POLLEN. Genetics 11: 163–199, illus.

(6) Collins, G. N.
1909. A NEW TYPE OF INDIAN CORN FROM CHINA. U. S. Bur. Plant Indus. Bul. 161, 30 pp., illus.

(7) Culpepper, C. W., and Magoon, C. A.
1924. STUDIES UPON THE RELATIVE MERITS OF SWEET CORN VARIETIES FOR CANNING PURPOSES AND THE RELATION OF MATURITY OF CORN TO THE QUALITY OF THE CANNED PRODUCT. Jour. Agr. Res. 28: 403–443, illus.

(8) ——— and Magoon, C. A.
1927. A STUDY OF THE FACTORS DETERMINING QUALITY IN SWEET CORN. Jour. Agr. Res. 34: 413–433, illus.

(9) Doxtator, C. W.
1937. STUDIES OF QUALITY IN CANNING CORN. Amer. Soc. Agron. Jour. 29: 735–753, illus.

(10) Haddad, E. S.
1931. MORPHOLOGICAL DEVELOPMENT OF SWEET CORN PERICARP IN TWO INBRED LINES AND THEIR F_1 HYBRID. Ind. Agr. Expt. Sta. Bul. 347, 24 pp. illus.

(11) Harrison, G. J.
1931. METAXENIA IN COTTON. Jour. Agr. Res. 42: 521–544, illus.

(12) Harrison, W.
1911. DIE FARBENREAKTIONEN DER STÄRKE UND IHRER HYDROLYTISCHEN SPALTUNGSPRODUKTE MIT JOD. Ztschr. f. Chem. u. Indus. Kolloide 9: 5–9.

(13) Heinze, P. H., and Murneek, A. E.
1940. COMPARATIVE ACCURACY AND EFFICIENCY IN DETERMINATION OF CARBOHYDRATES IN PLANT MATERIAL. Mo. Agr. Expt. Sta. Res. Bul. 314, 23 pp., illus.

(14) Lampe, L.
1931. A MICROCHEMICAL AND MORPHOLOGICAL STUDY OF THE DEVELOPING ENDOSPERM OF MAIZE. Bot. Gaz. 91: 337–376, illus.

(15) Meyer, A.
1886. UEBER STÄRKEKÖRNER, WELCHE SICH MIT JOD ROTH FÄRBEN. Deut. Bot. Gesell. Ber. 4: 337–362, illus.

(16) Nebel, B. R.
1930. XENIA AND METAXENIA IN APPLES. N. Y. State Agr. Expt. Sta. Tech. Bul. 170, 16 pp., illus.

(17) ———
1936. METAXENIA IN APPLES. Jour. Hered. 27: 345–349, illus.

(18) Nixon, R. W.
1928. IMMEDIATE INFLUENCE OF POLLEN IN DETERMINING THE SIZE AND TIME OF RIPENING OF THE FRUIT OF THE DATE PALM. Jour. Hered. 19: 241–255, illus.

(19) Randolph, L. F.
1936. DEVELOPMENTAL MORPHOLOGY OF THE CARYOPSIS IN MAIZE. Jour. Agr. Res. 53: 881–916, illus.

(20) Richardson, C.
1883. AN INVESTIGATION OF THE COMPOSITION OF AMERICAN WHEAT AND CORN. U. S. Bur. Chem. Bul. 1, 69 pp.

(21) Salisbury, J. H.
1849. HISTORY AND CHEMICAL INVESTIGATION OF MAIZE, OR INDIAN CORN. 206 pp., illus. Albany, N. Y.

(22) Schreiner, E. J., and Duffield, J. W.
1942. METAXENIA IN AN OAK SPECIES CROSS. Jour. Hered. 33: 97–98, illus.

(23) Smith, G. M.
1933. GOLDEN CROSS BANTAM SWEET CORN. U. S. Dept. Agr. Cir. 268, 12 pp., illus.

(24) Swingle, W. F.
1928. METAXENIA IN THE DATE PALM. POSSIBLY A HORMONE ACTION BY THE EMBRYO OR ENDOSPERM. Jour. Hered. 19: 257–268.

(25) Waller, A. E.
1917. XENIA AND OTHER INFLUENCES FOLLOWING FERTILIZATION. Ohio Jour. Sci. 17: 273–284.

(26) Weatherwax, P.
1922. A RARE CARBOHYDRATE IN WAXY MAIZE. Genetics 7: 568–572.

THE FERMENTATION OF CIGAR-LEAF TOBACCO AS INFLUENCED BY THE ADDITION OF YEAST[1]

By M. F. GRIBBINS, formerly *assistant in agricultural and biological chemistry*, D. E. HALEY, *professor of soils and phytochemistry*, and J. J. REID, *associate professor of bacteriology, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Tobacco of the cigar-leaf type must first undergo certain periods of fermentation before it can be used for cigar manufacture. The chemical changes that take place during fermentation involve, among others, a loss of some organic compounds and the synthesis of others with the final result that the aromatic and smoking qualities of the leaf are markedly improved.

Many theories have been advanced to explain the nature of the fermentation process. Among these may be mentioned the bacterial theory of Suchsland (*12*),[2] who claimed that certain micro-organisms have the power of acting upon leaf constituents and developing the characteristic flavor and aroma of fermented tobacco. The work of Behrens (*1*), Vernhout (*13*), and Koning (*4*) tends to support this theory.

Apparently, the first attempt to challenge the bacterial theory was made by Loew (*5*), who stated that leaf enzymes rather than bacteria provided the necessary mechanism of fermentation. For a long time this so-called enzyme theory of Loew's supplanted the bacterial theory. Recently, however, the work of Johnson (*2*) has revived the bacterial theory, and the later work of Reid and his coworkers (*6, 7, 8, 9, 10*) has proved conclusively that the major changes in fermentation are brought about through the activity of certain bacteria which usually develop in large numbers during the fermentation process, and that leaf enzymes play only a subordinate role.

The development of the micro-organisms of fermentation appears to be markedly affected by variations in the composition of the leaf. Some of these variations may occur during the curing process, but they may also be due in large measure to the environmental conditions under which the plants are grown, such as the nature of the soil, the season, and certain cultural practices which include the addition of fertilizers. In some cases the composition of the leaf may be of such a nature as to satisfy the nutrient requirements of undesirable rather than of desirable types of organisms. If, however, conditions are such as to favor the latter, the former do not appear

---

[1] Received for publication April 26, 1943. Paper No. 1151 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 381.

Journal of Agricultural Research, Washington, D. C.

Vol. 69, No. 9
Nov. 1, 1944
Key No. Pa.-70

to thrive. It seems obvious, therefore, that a stimulation of the desirable types of fermentation organisms should aid materially in obtaining a satisfactory fermentation, and for this reason an effort was made as herein reported to obtain such a condition by the addition of a material high in growth-accessory substances, namely, yeast, and to study the effect of such a treatment upon the microflora of the leaves during the preliminary stages of fermentation. No direct action of yeast on the fermentation process was expected as Street (*11*) had shown that yeast cells live but a short time after they are applied to leaf surfaces.

The use of yeast as a supplementary treatment of cigar-leaf tobacco prior to fermentation was first introduced by Koller (*3*) in 1858, and later was employed by other European workers. Recently, Street (*11*) reported on the use of yeast in the fermentation of domestic cigar-leaf tobacco. His results showed that the temperature rises in the earlier stages of fermentation were greater with all yeast treatments than when water alone was used and, furthermore, that yeast treatment produced a product which appeared to be more completely fermented in a given period of time as judged by appearance and smoking qualities than untreated samples. In his investigations, however, he gave no direct consideration to the effect of the treatment on the microflora of the leaves.

PLAN OF THE EXPERIMENTS AND MATERIALS USED

Preliminary experiments to determine the effect of yeast treatment on the fermentation of Pennsylvania cigar-leaf tobacco were first undertaken on representative samples of the 1940 crop. At the conclusion of these experiments similar studies were conducted on representative samples of the 1941 crop. For this work representative samples of freshly cured tobacco were obtained from Landisville and from the tobacco experimental laboratory at Roseville, Pa., on December 31, 1941.

The 1941 crop had not received sufficient rainfall for normal development and hence may be termed a "dry-weather" crop. Tobacco produced during a dry season is usually far more difficult to ferment than that produced under conditions of normal and well-distributed rainfall. Moreover, the 1941 tobacco did not cure well owing to the dry weather which prevailed after the crop was harvested.

In preparing samples for fermentation, five wooden cases, each 30 inches long, 9 inches wide, and 11.5 inches deep were used. Each case held 40 pounds of tobacco under 400-pound pressure. The yeast, when used, was applied to the samples in the form of a 10 percent aqueous suspension before the tobacco was placed in the case, and at the rate of 0.75 percent of the solid content of the weight of the tobacco. This quantity had been found in previous experiments to have no deleterious effect on the aroma of the tobacco. The yeast used represented a moist, freshly prepared, commercially cultivated strain of *Saccharomyces cerevisiae* of the type used in bread making.

EXPERIMENT 1

The treatments received by the tobacco both in the field and in the laboratory are presented in table 1. The tobacco in cases 4 and 5 came from five different experimental plots at the tobacco experimental laboratory, 8 pounds from each plot constituting the 40 pounds.

TABLE 1.—*Description of the tobacco samples used for fermentation studies (experiment 1)*

Sample No.	Case No.[1]	Where grown	Fertilizer treatment of crop		Supplementary laboratory treatment
			Pounds per acre	Formula	
1	1	Landisville	1,000	4-8-12	Control.
2	2	do	1,000	4-8-12	Yeast.
3	3	do	1,000	4-8-12	Do.
4	4	Roseville	1,000	3-9-24	Control.
5	5	do	1,000	3-9-24	Yeast.
6	4	do	1,000	3-9-12	Control.
7	5	do	1,000	3-9-12	Yeast.
8	4	do	1,000	3-6-12	Control.
9	5	do	1,000	3-6-12	Yeast.
10	4	do	1,000	3-9-18	Control.
11	5	do	1,000	3-9-18	Yeast.
12	4	do	1,000	3-12-12	Control.
13	5	do	1,000	3-12-12	Yeast.

[1] The tobacco used in cases 1 and 2 was from Kreider seed, that in case 3 from Kreider and Swarr seed mixed, and that in cases 4 and 5 from seed of a Swarr-Hibshman strain.

The tobacco was uniformly sprayed with water or yeast suspension and then exposed to the air for 24 hours. At the end of that period samples were taken for biological analysis. The results, representing the average of at least three samples in every case, are presented in table 2. In these and similar studies the bacteriological technique employed was that described by Reid, McKinstry, and Haley (*9*).

TABLE 2.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 1)*

Sample No.	Organisms per gram of tobacco			Nature and distribution of bacteria		
	Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Rods	
					Gram−	Gram+
	Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Perent*
1	0.9	80	20	55	45	0
2	4.0	242	3,500	84	16	0
3	1.1	26	2,800	100	0	0
4	3.1	66	95	100	0	0
5	.2	34	1,300	0	50	50
6	.6	72	20	0	100	0
7	2.0	95	5,200	50	50	0
8	.1	50	30	0	100	0
9	7.0	57	13,200			
10	.3	32	40	0	50	50
11	4.0	67	16,300	0	33	67
12	.7	72	260	0	50	50
13	3.0	30	7,900	33	33	33

When the tobacco was placed in the fermentation chamber, a thermometer was inserted in the center of each case. The centers of cases 4 and 5 were occupied by samples 8 and 9, respectively. The changes in temperature noted are presented in table 3. An effort was made to obtain an environmental temperature of 72° F. at first, but certain mechanical difficulties were experienced before this temperature could be attained and stabilized.

A humidity of 75 percent was maintained for the first 27 days of fermentation. At the end of that period the temperature was increased approximately 20° F., and the humidity was raised to 80 percent. On the 71st day of fermentation, the temperature was lowered to approximately 80° F. and the humidity to 75 percent.

On the 93d day the tobacco was removed from the cases and aerated. Samples were taken at that time from each case for obtaining the judgment of a leaf expert in respect to its quality and the extent of this preliminary phase of fermentation. The remainder was remoistened and an effort was made to approximate 32 percent moisture in all samples. The tobacco was then placed in the original cases and returned to the fermentation chamber.

TABLE 3.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 1)*

Interval (days)	Room temperature	Degrees above room temperature in—				
		Case 1	Case 2	Case 3	Case 4	Case 5
	°F.	*°F.*	*°F.*	*°F.*	*°F.*	*°F.*
1	75	7	9	6	6	15
2	68	13	20	14	5	16
3	72	7	14	12	6	18
4	72	6	16	15	6	18
5	72	6	15	16	6	18
6	72	6	15	16	8	17
7	72	6	14	17	10	18
8	72	6	13	17	11	18
	72	6	12	17	11	18
10	72	6	11	16	13	16
12	73	6	8	14	15	15
14	71	6	8	13	16	14
16	71	6	7	11	14	12
18	72	4	5	7	11	9
20	72	4	4	6	7	6
25	71	4	4	3	5	7
30	92	1	3	3	5	6
40	89	4	4	3	5	7
50	89	4	3	6	5	8
60	90	3	2	4	4	5
70	91	2	1	3	4	4
80	80	1	1	2	4	3
90	80	0	1	1	3	3
100	80	6	6	2	9	6
110	80	5	6	2	6	4
120	80	5	5	2	4	4

When the tobacco was removed from the cases and reconditioned following the 92d day of fermentation, an opportunity was afforded for studying the microflora of the individual samples listed in table 1. The results obtained are presented in table 6.

TABLE 4.—*Moisture content of the tobacco samples before and during fermentation (experiment 1)*

Case No.	Moisture content after fermentation—interval (days)					
	0	12	19	54	70	92
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	29.3	29.2	29.2	29.0	30.7	27.3
2	35.0	33.4	32.1	35.1	33.7	32.9
3	29.2	28.1	27.0	32.8	31.3	30.1
4	22.2	22.1	22.0	23.8	26.0	25.5
5	22.4	22.3	22.2	24.3	28.8	28.9

TABLE 5.—*Number and kind of organisms on samples of tobacco taken from cases at different intervals during the fermentation period (experiment 1)*

Interval (days)	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms	Rods	
							Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
12	1	180	300	---	100	---	---	---
	2	2,390	500	1,000	100	---	---	---
	3	1,250	760	1,100	---	---	100	---
19	1	110	18	---	100	---	---	---
	2	2,400	49	1,000	100	---	---	---
	3	95	6,500	500	87	---	4	9
27	3	21	480	4,500	40	60	---	---
	4	4	285	2,200	44	28	---	28
	5	5.2	800	4,000	100	---	---	---
35	1	1,900	6	0	79	21	---	---
	2	1,500	3	22	79	21	---	---
	3	330	780	800	95	5	---	---
	4	4.6	25	110	90	10	---	---
	5	5.4	3,400	2,000	100	0	---	---
54	1	1,230	4	0	100	0	---	---
	2	410	.8	3	84	16	---	---
	3	820	37	12	84	16	---	---
	4	5	9	1,800	100	0	---	---
	5	700	270	40	89	11	---	---
70	1	995	7	60	91	9	---	---
	2	560	(1)	30	100	0	---	---
	3	1,100	6	3	95	5	---	---
	4	1,300	30	67	91	9	---	---
	5	2,110	21	680	95	5	---	---

[1] Trace.

Samples of tobacco were taken by means of borings made into the center of the cases as the fermentation progressed. Moisture determinations were made on these samples before they were placed in the case, and on the 12th, 19th, 54th, 70th, and 92d day of fermentation. The results obtained are given in table 4.

Samples removed from cases at different intervals were analyzed to determine the number of organisms and the nature of those predominating. The results are given in table 5.

TABLE 6.—*Number and kind of organisms on the leaves of the 13 original samples of tobacco at the end of the 92d day of fermentation*

Sample No.	Case No.	Supplementary treatment	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
			Million	*Thousand*	*Thousand*	*Percent*	*Percent*
1	1	Control	590	<1	<1	87	13
2	2	Yeast	720	1	<1	63	37
3	3	do	740	1	<1	93	7
4	4	Control	220	1	310	93	7
5	5	Yeast	930	1	<1	87	13
6	4	Control	1,060	1	28	87	13
7	5	Yeast	420	1	<1	80	20
8	4	Control	680	1	75	63	37
9	5	Yeast	510	1	<1	87	13
10	4	Control	1,380	1	570	87	13
11	5	Yeast	650	1	96	93	7
12	4	Control	670	1	107	100	0
13	5	Yeast	1,960	1	<1	80	20

Tests conducted prior to fermentation indicated a good burn in the tobacco making up cases 1, 2, and 3; the burn of the tobacco in cases 4 and 5 was classed as fair. In every instance, however, the aroma was classed as poor.

The samples of tobacco taken at the end of the 92d day of fermentation and submitted to the judgment of an expert on cigar-leaf tobacco yielded the results shown in table 7.

TABLE 7.—*Burning quality, aroma, and fermentation of samples of tobacco on the 92d day of fermentation, as judged by an expert on cigar-leaf tobacco*

Case	Burn	Aroma	Degree of fermentation
1	Good	Poor	Not pronounced.
2	do	Fair	Fair to good.
3	do	Very mild	Very good; best of all samples tested.
4	Fair	Poor	Not pronounced.
5	Good	Good	Good; second only to sample 3.

EXPERIMENT 2

At the conclusion of the first experiment a second and somewhat similar experiment was instituted. For this experiment two cases were filled with cured tobacco from the southern part of Lancaster County and two with tobacco from the laboratory at Roseville. The Roseville tobacco came from duplicates of the plots from which the tobacco used in cases 4 and 5 of experiment 1 had been obtained. All these samples had been kept in bales for 4 months longer than those of experiment 1.

An effort was made to approximate a 28-percent moisture content and to maintain a humidity of 75 percent and a temperature in the fermentation chamber of 80° F. The experiment was started on May 2, 1942. As in the first experiment, samples were taken for analysis after the yeast and moisture treatment and before the tobacco was placed in the cases.

A description of the samples is given in table 8 and the results of the initial study of leaf organisms are presented in table 9. The temperature changes as influenced by yeast treatment are shown in table 10.

TABLE 8.—*Description of the tobacco samples used for fermentation studies (experiment 2)*

Sample No.	Case No.	Fertilizer treatment of crop		Supplementary laboratory treatment
		Pounds per acre	Formula	
20	11	1,000	4- 8-12	Control.
21	12	1,000	4- 8-12	Yeast.
22	13	1,000	3- 9-18	Control.
23	14	1,000	3- 9-18	Yeast.
24	13	1,000	3-12-12	Control.
25	14	1,000	3-12-12	Yeast.
26	13	1,000	3- 9-24	Control.
27	14	1,000	3- 9-24	Yeast.
28	13	1,000	3- 6-12	Control.
29	14	1,000	3- 6-12	Yeast.
30	13	1,000	3- 9-12	Control.
31	14	1,000	3- 9-12	Yeast.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis (experiment 2)*

Sample No.	Case No.	Organisms per gram of tobacco			Nature and distribution of bacteria			
		Bacteria	Molds	Yeasts and yeastlike organisms	Cocci	Coccoid forms	Gram−	Gram+
		Million	*Thousand*	*Thousand*	*Percent*	*Percent*	*Percent*	*Percent*
20	11	0.8	91	1	83	17		
21	12	2.2	950	520	91	9		
22	13	.8	9	53	100			
23	14	7.7	110	4,900	100			
24	13	.7	540	470	80		10	10
25	14	.7	131	1,400	100			
26	13	1.2	25	760	90			10
27	14	1.3	410	3,200	100			
28	13	.3	13	15	100			
29	14	9.2	58	8,400	100			
30	13	.1	30	36	100			
31	14	.7	90	4,100	100			

TABLE 10.—*Relation of yeast treatment to the temperature changes of fermenting cigar-leaf tobacco (experiment 2)*

Interval (days)	Room temperature	Degrees above room temperature in—			
		Case 11	Case 12	Case 13[1]	Case 14[1]
	°F.	*°F.*	*°F.*	*°F.*	*°F.*
1	81	3	11	4	4
2	81	5	12	4	4
3	80	7	15	6	11
4	80	8	16	6	13
5	80	9	17	6	14
6	80	9	17	6	14
7	80	9	16	7	13
8	80	9	15	7	11
9	80	9	12	6	10
10	80	8	10	5	7
12	81	7	9	5	6
14	80	7	9	6	6
16	80	7	8	5	5
18	80	5	6	4	5
20	80	5	6	4	5
25	80	5	6	4	5
30	80	5	7	4	5
40	80	5	7	5	5
50	81	3	4	2	2
60	80	3	3	3	1
70	80	2	2	2	0
80	80	2	1	1	0
90	80	2	1	1	0
100	80	1	0	1	0
110	80	0	0	0	0
120	80	0	0	0	0

[1] The center of cases 13 and 14 was occupied by samples 26 and27 respectively.

Samples were removed from the four cases on the 18th and 38th day of fermentation and were analyzed to determine the number of organisms and the nature of those predominating. The results are presented in table 11.

TABLE 9.—*Microflora of the tobacco before fermentation, moisture-free basis cases at different intervals during the fermentation period* (*experiment 2*)

Interval (days)	Case No.	Moisture	Organisms per gram of tobacco			Nature and distribution of bacteria	
			Bacteria	Molds	Yeastlike organisms	Cocci	Coccoid forms
		Percent	*Million*	*Thousand*	*Thousand*	*Percent*	*Percent*
18	11	24.2	2.4	68	<1	93	7
	12	25.9	600	7	8,400	87	13
	13	24.7	400	20	4,000	100	0
	14	28.0	2,700	27	5,100	93	7
38	11	25.0	1,270	<1	210	100	0
	12	28.1	2,900	13	8	100	0
	13	23.7	1,390	4	109	100	0
	14	30.8	1,790	<1	3	73	27

Tests conducted before fermentation showed that the tobacco making up cases 11 and 12 possessed a good burn but poor aroma while the tobacco making up cases 13 and 14 possessed a fair burn and poor aroma. Examination of these particular samples, after 120 days of fermentation, indicated an improvement in both burn and aroma as a result of the yeast treatment, paralleling the results obtained in experiment 1.

DISCUSSION OF RESULTS

The addition of yeast to cigar-leaf tobacco under the conditions of the experiments herein described was reflected in a relatively high initial increase in temperature during the preliminary stages of fermentation, as indicated in tables 3 and 10.

An examination of the samples receiving yeast showed the presence of a number of true yeasts 24 hours after treatment, but no other occasion of sampling was the presence of these organisms noted. On all samples, however, the presence of certain yeastlike organisms was observed, whether or not the samples were treated with yeast.

The number of these organisms was definitely increased as a result of yeast treatment, which appeared to be beneficial to the fermentation process. Entirely different yeastlike organisms were first noted on samples taken from the cases used in experiment 1 at the end of the 92d day of fermentation. In this instance, however, the addition of yeast, on the whole, appeared to be antagonistic to the development of these particular organisms.

The experiments to determine the influence of yeast treatment on the bacterial flora of the tobacco samples yielded somewhat erratic results. Owing to the differences ordinarily prevailing in the chemical composition of the leaves and the difficulties encountered in obtaining uniform samples, this was to be expected. On the whole, however, the addition of yeast resulted in an increase in the number of bacteria during the preliminary stages of fermentation. With but few exceptions, a high bacterial count was paralleled by a low mold content, which is considered highly desirable. There appeared to be little, if any, increase in mold development as a result of yeast treatment.

SUMMARY AND CONCLUSIONS

The results of fermentation studies dealing with the application of bakers' yeast to Pennsylvania cigar-leaf tobacco of the 1940 and 1941 crop are presented. The following conclusions seem justified:

(1) Initial temperature gains were greater with all yeast-treated tobacco under the conditions of these experiments.

(2) The addition of yeast stimulated the development of organisms considered necessary to the fermentation process.

(3) A more rapid initial fermentation was evidenced in the case of the yeast-treated samples.

LITERATURE CITED

(1) BEHRENS, J.
1896. DIE BEZIEHUNGEN DER MIKROORGANISMEN ZUM TABAKBAU UND ZUR TABAKFABRIKATION. Centbl. f. Bakt. [etc.] Abt. (II) 2: 514–527, 540–545.

(2) JOHNSON, J.
1934. STUDIES ON THE FERMENTATION OF TOBACCO. Jour. Agr. Res. 49: 137–160, illus.

(3) KOLLER, J. B. C.
1858. DER TABAK IN NATURWISSENSCHAFTLICHER, LANDWIRTHSCHAFTLICHER U. TECHNISCHER BEZIEHUNG. 134 pp. Augsburg.

(4) KONING, C. J.
1900. DER TABAK; STUDIEN ÜBER SEINE KULTUR UND BIOLOGIE. 86 pp., illus. Amsterdam and Leipzig.

(5) LOEW, O.
1899. CURING AND FERMENTATION OF CIGAR LEAF TOBACCO. U. S. Dept. Agr. Rpt. 59, 34 pp.

(6) MCKINSTRY, D. W., HALEY, D. E., and REID, J. J.
1938. A BACTERIOLOGICAL STUDY OF THE BULK FERMENTATION OF CIGAR LEAF TOBACCO. (Abstract) Jour. Bact. 35: 71.

(7) REID, J. J., HALEY, D. E., MCKINSTRY, D. W., and SURMATIS, J. D.
1937. THE RELATION OF CATALASE ACTIVITY TO THE MICROFLORA OF CURED AND FERMENTING TOBACCO. (Abstract) Jour. Bact. 34: 460.

(8) ——— MCKINSTRY, D. W., and HALEY, D. E.
1937. THE FERMENTATION OF CIGAR LEAF TOBACCO Science 86: 404.

(9) ——— MCKINSTRY, D. W., and HALEY, D. E.
1938. STUDIES ON THE FERMENTATION OF TOBACCO. 1. THE MICROFLORA OF CURED AND FERMENTING CIGAR LEAF TOBACCO. Pa. Agr. Expt. Sta. Bul. 356, 18 pp., illus.

(10) ——— MCKINSTRY, D. W., and HALEY, D. E.
1938. STUDIES ON THE FERMENTATION OF TOBACCO. 2. MICROÖRGANISMS ISOLATED FROM CIGAR LEAF TOBACCO. Pa. Agr. Expt. Sta. Bul. 363, 18 pp., illus.

(11) STREET, O. E.
1938 THE ROLE OF YEASTS IN THE FERMENTATION OF TOBACCO. 1. PRELIMINARY STUDIES. Conn. Agr. Expt. Sta. Bul. 410, pp. 414–443, illus.

(12) SUCHSLAND, E.
1891. UEBER TABAKSFERMENTATION. Deut. Bot. Gesell. Ber. 9: 79–81.

(13) VERNHOUT, J. H.
1899. ONDERZOEK OVER BACTERIËN BIJ DE FERMENTATIE DER TABAK. Buitenzorg Meded. s' Lands Plantentuin 34, 49 pp., illus.

VALUE OF SOYBEAN MEAL PREPARED FROM DAMAGED (BIN-BURNED) SOYBEANS AS A FEED FOR GROWING SWINE[1]

By J. L. KRIDER, *assistant chief in swine husbandry*, B. W. FAIRBANKS, *chief in swine husbandry*, and W. E. CARROLL, *head, Department of Animal Husbandry, Illinois Agricultural Experiment Station*[2]

INTRODUCTION

In Illinois during the spring and summer of 1942, many soybeans came out of storage in a badly damaged or "bin-burned" condition caused by an excess moisture. A sample of soybean meal was prepared by the expeller method from some of these damaged soybeans for comparison with normal expeller soybean meal prepared from mature soybeans that appeared sound after storage. The soybeans from which the bin-burned meal used in this test was prepared were described as "average 75 to 80 percent damaged."[3] At the Illinois station in the fall of 1942, a study was made of the feeding value of the bin-burned soybean meal in comparison with normal expeller soybean meal for growing-fattening pigs in dry lot.

METHODS

The method used consisted of feeding the pigs by the paired feeding technique for equal gains in order to obtain quantitative results. The writers believe this is the first time that paired feeding for equal gains has been attempted with swine. One pig of each pair received the check ration which contained normal soybean meal, while the other pig was fed the ration which contained bin-burned soybean meal.

The pigs were paired on the basis of weight, litter, sex, breed, type, condition, and probable outcome. Ten pairs of pigs were fed. They were purebred Chester Whites, Duroc Jerseys, Hampshires, and Poland Chinas raised on the University farm. Their weights varied from 28 to 80 pounds at the beginning of the test. The youngest pairs were 51 to 56 days old when the test began.

The basal ration used consisted of ground yellow corn, soybean meal, alfalfa meal, and mineral mixture. The mineral mixture was composed of 2 parts of ground limestone, 2 parts of steamed bonemeal, and 1 part of salt. The proportion of corn and soybean meal in the mixture was varied as the pigs increased in weight, whereas the alfalfa meal was fed at the 10-percent level and the mineral mixture was fed at the 2-percent level throughout the experiment. The changes made are indicated in table 1.

---

[1] Received for publication May 25, 1943.

[2] The assistance of R. H. McDade, chief swine herdsman, is gratefully acknowledged.

[3] "Bin-burned" soyean meal was prepared and donated to the University of Illinois by the Central Soya Company, Inc., Gibson City, Ill.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 9
Nov. 1, 1944
Key No. Ill.-79

TABLE 1.—*Variations in the proportion of the ingredients of the rations fed the growing swine at different live weights*

Feeds	Proportion in which the feeds were mixed for pigs weighing—					
	75 pounds or less		75 to 125 pounds		Over 125 pounds	
	A[1]	B[2]	A[1]	B[2]	A[1]	B[2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ground yellow corn	55	58	63	66	68	70
Normal soybean meal	33		25		20	
Bin-burned soybean meal		30		22		18
Alfalfa meal	10	10	10	10	10	10
Steamed bonemeal	.8	.8	.8	.8	.8	.8
Ground limestone	.8	.8	.8	.8	.8	.8
Sodium chloride	.4	.4	.4	.4	.4	.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Normal expeller soybean-meal mixture (check pigs).
[2] Bin-burned expeller soybean-meal mixture (test pigs).

The alfalfa meal was fed at the 10-percent level to insure against a possible vitamin deficiency in the all-vegetable diet. Recent experiments by the present writers [4] and by Ross, Phillips, Bohstedt, and Fargo at the Wisconsin station [5] indicate that higher levels of alfalfa meal than the usual 5 percent may be beneficial.

The chemical composition of the soybean meals used in the experiment, determined upon composite samples, is given in table 2.

TABLE 2.—*Chemical composition of the soybean meals on the fresh basis*

Feeds	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Normal soybean meal	88.10	42.50	4.27	5.04	4.86	31.43
Bin-burned soybean meal	93.41	46.00	4.60	5.60	6.34	30.87

In preparing the feed mixtures, the higher protein content of the bin-burned soybean meal was taken into consideration by equalizing the total percent of crude protein in the mixtures for the pigs in each weight group. As the pigs increased in weight, the ground corn and soybean meal were varied so that the following percentages of crude protein were fed: For pigs weighing 75 pounds or less, 20.3 percent; 75 to 125 pounds, 17.5 percent; over 125 pounds, 16.0 percent.

The pigs were fed twice daily in individual feeding crates. The feed mixture was weighed to one-tenth of a pound and fed in a metal trough with which each crate was equipped. A small amount of water was poured on the feed to prevent waste and to induce consumption. The pigs were watched closely in an attempt to keep the feed consumption up to the limit of the gains of the slowest-gaining pig of each pair. Records were kept of feed refusals. Individual weights of all pigs were taken at weekly intervals and the feed allowances for the following week were adjusted according to the gains made during the previous week.

---

[4] Unpublished data.

[5] WISCONSIN AGRICULTURAL EXPERIMENT STATION. USE 15% ALFALFA IN SOYBEAN OILMEAL PIG RATIONS. Wis. Agr. Expt. Sta. Bul. 453, pp. 18-20, illus. 1941.

WISCONSIN AGRICULTURAL EXPERIMENT STATION. PIGS PAY HIGH RETURNS ON 15% ALFALFA. Wis. Agr. Expt. Sta. Bul. 455, pp. 8-10, illus. 1942.

The pigs were kept on concrete floors, the control pigs in one group and the pigs receiving the bin-burned soybean meal in another. The four small pigs of each group were also separated from the six larger pigs of the group. Each subgroup was given a pen in the central swine barn opening onto an outside concrete runway to which the pigs had access.

The experiment began on September 8, 1942, and was discontinued after 134 days, but before the pigs reached market weight, because the supply of bin-burned meal was exhausted.

RESULTS

The results of this experiment are summarized in table 3.

Table 3 shows that in 5 of the 10 pairs the test pig required more feed per pound of gain, while in 4 pairs the check pig required more feed. In 1 pair, the feed required for a unit of gain was the same. These results on economy of gain are too nearly a chance distribution to indicate any significant differences in the feeding value of the two rations. This was verified by statistical treatment of the data by Student's method with argument *t* as described by Snedecor.[6]

As would be expected, there was considerable variation in the economy of the gains among the various pairs. The largest differences in economy of gains existed between the pigs of pair 1 and pair 2. In these two pairs the check pigs made a unit of gain on 16.3 percent and 16.2 percent less feed respectively, than the test pigs.

In paired feeding for equal gains, it is not surprising that the average daily gains are less than the feed mixtures are capable of supporting under conditions of unrestricted feeding. Severe winter weather during the last 60 days of the test may have contributed to the slow gains. The check pig in pair 4, the pair with the lowest average daily gains, often had digestive disturbances, and also had more feed refusals than any other pig on test. This in part explains the small gains of the pigs in pair 4.

A record of feed refusals indicated that the feed mixtures were equally palatable.

The ideal in paired feeding for equal gains would be to have both pigs of a pair gain exactly the same weight every week. Since pigs vary greatly, in eating habits and other traits, even when strict paired methods are used, it is not surprising that weekly gains varied considerably. This variability may be noted in table 3. With 10 pairs of pigs fed for 19 weeks, a total of 190 weekly comparisons between the gains of pair mates is possible. Of the 190 weekly comparisons between gains of pair mates, 34 gave identical gains, while in 74 the check pigs gained more and in 82 the weekly gain favored the pigs fed bin-burned soybean meal. The deviation of 4 from the ideal of a chance distribution is considerably less than the standard deviation, and hence is not significant. When Student's method with argument *t* was calculated as described by Snedecor for weekly gains on each pair of pigs for the 19 weeks, the *t* values were very small and no statistically significant differences were noted. This indicates that the method of feeding for equal gains was successful.

---

[6] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Rev. ed., 388 pp., illus. Ames, Iowa. 1938.

TABLE 3.—*Weights, gains, and feed consumption of 10 pairs of pigs, 1 of each pair being on the check ration (A) and the other on the bin-burned soybean-meal ration (B)*

Item	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		Pair 6		Pair 7		Pair 8		Pair 9		Pair 10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Final weight......pounds..	156	147	118	125	99	105	95	94	158	163	181	181	178	168	114	124	179	184	161	164
Initial weight........do....	49	42	28	34	32	33	42	40	52	55	67	69	70	59	42	49	76	80	72	70
Total gain...........do....	107	105	90	91	67	72	53	54	106	108	114	112	108	109	72	75	103	104	89	94
Period on test........days..	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134	134
Average daily gain pounds..	.80	.79	.67	.68	.50	.54	.40	.40	.79	.81	.85	.84	.81	.81	.54	.56	.77	.78	.66	.70
Gain or loss in pounds after week No.:																				
1	1	−1	1	0	1	0	1	2	4	0	3	3	4	2	3	1	5	2	4	0
2	4	1	2	2	0	2	1	2	2	4	7	5	3	4	3	3	2	3	−1	2
3	4	3	4	2	4	1	1	1	3	2	1	−2	3	3	0	−2	4	5	3	3
4	4	3	0	1	3	2	1	1	6	2	5	6	6	8	2	5	6	6	7	7
5	6	5	4	5	3	5	1	4	5	8	7	7	8	6	4	7	8	7	3	5
6	3	6	6	2	4	3	3	0	2	3	5	5	5	5	5	3	0	3	5	0
7	3	5	2	5	2	4	2	4	4	5	5	4	5	7	6	6	6	7	6	7
8	7	3	6	5	7	3	1	2	6	5	4	4	9	10	5	5	8	8	5	4
9	5	6	6	5	2	6	2	1	5	5	5	7	3	3	8	6	4	3	3	6
10	2	5	7	6	4	6	1	4	7	7	9	7	14	9	4	9	8	9	8	1
11	2	4	1	4	0	−1	0	2	4	4	0	6	−1	5	−2	−3	1	6	4	7
12	8	10	7	8	4	7	7	3	7	8	10	6	7	8	5	4	7	4	1	1
13	10	5	7	4	6	5	6	2	8	6	4	7	7	5	3	4	7	4	0	6
14	8	8	9	7	7	5	2	5	8	8	10	13	10	13	9	9	12	10	8	8
15	10	6	6	6	6	5	5	4	7	8	11	6	9	8	7	9	1	8	8	9
16	0	1	6	6	5	7	4	3	1	2	0	2	−6	−5	−3	−6	9	1	11	11
17	9	12	12	13	3	3	10	9	8	8	5	7	7	2	7	10	7	7	8	1
18	6	8	2	1	3	3	2	1	8	10	10	7	5	7	3	3	1	3	4	6
19[1]	15	15	2	9	3	6	3	4	11	13	13	12	10	9	3	2	7	8	2	10
Total feed eaten...pounds..	351.5	412.5	283.9	342.1	259.8	279.7	248.5	227.7	416.3	464.2	443.9	487.5	513.9	497.7	347.0	345.0	502.7	488.2	388.8	429.8
Average ration.......do....	2.62	3.08	2.12	2.55	1.94	2.09	1.85	1.70	3.11	3.46	3.31	3.64	3.84	3.71	2.59	2.57	3.75	3.64	2.90	3.21
Feed consumed per pound of gain..........pounds..	3.29	3.93	3.15	3.76	3.88	3.88	4.69	4.22	3.93	4.30	3.89	4.35	4.76	4.57	4.82	4.60	4.88	4.69	4.37	4.57

[1] 8 days in this period. Initial and final weights are the averages of 3 weights taken on consecutive days. Single weights were used in the other cases.

SUMMARY

When a feed mixture of ground yellow corn, normal expeller soybean meal, alfalfa meal, steamed bonemeal, limestone, and sodium chloride was compared with a similar feed mixture in which expeller soybean meal prepared from bin-burned soybeans was fed to growing pigs in place of normal soybean meal, both mixtures were consumed readily. The paired feeding method for equal gains was used in this trial, which involved 10 pairs of young pigs. In 5 pairs of pigs, the check mate made more economical gains as measured by feed eaten per pound of gain. In 4 pairs, the mate fed bin-burned soybean meal gained more economically, while in 1 pair the economy of gains was the same. These results are too nearly a chance distribution to indicate any statistically significant differences in the feeding value of the two feed mixtures. From these data, it may therefore be concluded that the soybean meal prepared from the damaged soybeans was equal in feeding value to the soybean meal prepared from sound soybeans for growing-fattening pigs in dry lot.

○

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., NOVEMBER 15, 1944 NO. 10

BIONOMICS OF THE LYMNAEID SNAIL, STAGNICOLA BULIMOIDES TECHELLA, THE INTERMEDIATE HOST OF THE LIVER FLUKE IN SOUTHERN TEXAS[1]

By O. WILFORD OLSEN

Associate parasitologist, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

The liver fluke (*Fasciola hepatica* Linn.) is one of the most common and economically important parasites of cattle and sheep in the Gulf coast region of the United States. Slaughter records for 10 years under Federal meat inspection, obtained from six Gulf coast packing houses in Texas, Louisiana, and Florida, show 37.5 percent of the native adult cattle and 6 percent of the calves to be infected with the liver fluke. This condition results in the loss of the liver as an item of food, together with many unthrifty cattle and some death losses. Although the economic significance of this parasite and its general distribution in Texas were reported in 1891 by Francis (*4*),[2] it was not until about 40 years later that Sinitsin (*14, 15*) demonstrated that *Stagnicola bulimoides techella* (Hald.), a common fresh-water snail, was the intermediate host of the liver fluke in the coastal region of Louisiana and Texas. A prerequisite in the planning of effective control measures against this snail is a knowledge of its bionomics.

REVIEW OF WORK OF PREVIOUS INVESTIGATORS

A study of the literature shows that the bionomics of the principal snail intermediate hosts of the liver fluke in different parts of the world depends on the species involved and the localities in which they are found.

In the British Isles, *Galba truncatula* (Müller), the common European intermediate host of the liver fluke, was found by Walton (*23*) to be active throughout the year, producing two generations annually during seasons of normal precipitation and three generations during wet periods. According to Peters (*10*), the snails showed a preference for regions having clay soils over which water moved slowly and were found rarely in streams, even during drought. Walton and Wright (*24*) found these snails in water having a pH of 6.0 to 8.6. They concluded that the hydrogen-ion concentration of the water was of little importance in determining the distribution of the snails.

In Germany, Mehl (*9*) classified the habitats of *Galba truncatula* into three types, depending on the prevalence of water in each. They were as follows: (1) Streams and springs, where frost, heat, and drought did not affect the snails and in which the water was rich in oxygen, thus maintaining large populations throughout the year

---

[1] Received for publication May 24, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 402.

Journal of Agricultural Research, Washington, D. C.

Vol. 69, No. 10
Nov. 15, 1944
Key No. A-219

under conditions favorable for continuous breeding; (2) roadside excavations and drainage and irrigation ditches that became dry during normal summers but might retain water during rainy years; and (3) meadows and flood plains on which rainfall, overflows, or irrigation provided favorable conditions for the snails. After droughts that destroyed the snail populations, high water and irrigation restocked these last-mentioned areas from the stream beds. The snails were observed to have a longevity of 10 to 17 months. They were capable of surviving desiccation as long as 4½ months, depending on temperature, soil type, available shade, depth to which the snails entered the soil, and the manner in which the shell orifice was closed.

In Australia, Ross and McKay (*11*) found *Limnea brazieri* (Smith), the intermediate host of *Fasciola hepatica*, in shallow pools, on mud, on sandy and rocky bottoms, in deep water, and clinging to stones in swiftly running rivers. *L. brazieri* was unable to withstand desiccation longer than 24 hours. Under natural conditions, longevity appeared to be about 12 months. Reproduction occurred, generally, during spring and summer although it was more or less continuous throughout the year but on a lower level.

In the Philippine Islands, *L. philippinensis* Nevill, the intermediate host of *Fasciola gigantica* Cobbold and *F. hepatica*, was destroyed in the field by a drought of 3 months' duration, even though the soil did not become dry immediately after cessation of rain, according to De Jesus and Mallari (*8*) and De Jesus (*7*). The snails lived as long as 94 to 110 days in the laboratory.

In the Hawaiian Islands, Alicata (*1*) found that *Fossaria ollula* (Gould), the intermediate host of *Fasciola gigantica*, bred throughout the year under natural conditions. The snails generally inhabited the shallow, lowland marshes but were found occasionally in swiftly flowing streams. Their longevity in the laboratory was 114 to 164 days. It was reported also by Alicata, Swanson, and Goo (*2*) that complete drainage would kill all snails and their eggs in about 5 days. The range of *F. ollula* includes both China and Japan, from which they were introduced into Hawaii.

In Puerto Rico, Van Volkenberg (*21*, *22*) found *Lymnaea cubensis* Pfr., the intermediate host of *Fasciola hepatica*, living in stagnant water of swampy regions, in shallow sluggish streams, and in drainage ditches but not in swiftly running water or on gravelly stream beds.

In Canada, *Fossaria parva* (Lea) and *Stagnicola palustris nuttalliana* (Lea), the intermediate hosts of *Fascioloides magna* (Bassi), were found by Swales (*16*) to have different environments. *Fossaria parva* preferred areas that were wet and swampy in spring but completely dry during summer and autumn. The snails were very active and were frequently found on the mud. Survival during drought apparently took place in the soil, where the young snails were found during July. *S. palustris nuttalliana*, on the other hand, preferred permanent or semipermanent bodies of stagnant water containing much vegetation. The snails remained in the water and did not move out on the mud. The alkalinity of the water appeared not to influence the distribution of the snails.

In the United States, Francis (*4*), as already indicated, was the first investigator to point out the economic importance of the liver fluke in Texas. He recognized the moist, low-lying coastal plain as being the region of endemic fascioliasis, exceptions being the salt marshes where cattle were said to be relatively free of the parasite. However, he made no mention of the snail intermediate host in the region where he had conducted his investigations for a period of 3 years. He pointed out that fascioliasis occurred in cattle only when they had access to stagnant ponds that contained cercariae or to grass grown in wet places. Many years after the report of Francis on the liver fluke, Sinitsin (*15*) showed *Galba bulimoides techella* to be the common intermediate host of the liver fluke in Texas. He found the snails living on the muddy borders of both natural and artificial bodies of water.

The first natural infections of North American snails with *Fasciola hepatica* were reported independently by Shaw and Simms (*12*), in Oregon, for *Lymnaea* (*Galba*) *bulimoides* Lea, and by Sinitsin (*14*), in Louisiana, for *Galba bulimoides techella* (Hald.). These investigators verified the correctness of their observations by feeding the metacercariae to experimental animals in which infections with *F. hepatica* were obtained. In Oregon, Shaw and Simms (*13*) found the snails only in fresh water, the principal habitats being springs, seepages, and the banks of small streams. The snails were found at altitudes from near sea level to 5,600 feet, where the temperature was known to reach −40° F.

Fossaria parva (Lea) was reported by Hoff (*5, 6*) to produce but a single generation per year in Illinois, whereas Van Cleave (*19, 20*) found that *F. modicella* produced two generations annually in Indiana. *F. modicella*, the intermediate host of the liver fluke in Utah, was found to be very resistant to freezing as it went into hibernation when the temperature fell to 40° F. (*17, 18*).

In view of these differences in the bionomics of the snail intermediate hosts of the liver fluke, it was considered desirable to make a detailed study of the bionomics of *Stagnicola bulimoides techella* in southern Texas. The results are reported in the present paper.

SCOPE AND METHODS OF WORK

Field investigations were made in 1940 and 1941 in the Gulf coast prairie, which is the upper part of the Gulf coast region of Texas, to determine the principal types of habitats of the snails, to study their life history during the different seasons, and to obtain data on reproduction, survival, longevity, dissemination, and natural infection. The work was carried on during all the seasons and under conditions ranging from extreme drought to abnormally high precipitation. Laboratory studies were conducted simultaneously to verify some of the observations and also to provide information that could not be obtained readily in the natural habitat of the snails.

The life cycle and number of generations of the snails were determined under natural conditions by making frequent collections and observations in a series of habitats. The snails obtained from these places were measured and then divided into adult and juvenile classes based on average size at which maturity occurred, as predetermined in laboratory cultures. The percentage of juvenile snails found during the various months or seasons indicated the appearance of a new generation or the maturation of a previous one.

In the life-cycle studies in the laboratory, cultures of snails were kept in large, moist chamber dishes. The snails lived and reproduced most successfully when the bottoms of the dishes were covered with mud, part of which was exposed above the shallow water. Filamentous green algae provided the most suitable type of food.

Data on survival during drought were obtained in the field by collecting estivating snails from the dry soil of the habitats at different periods between the time the pools dried and the reappearance of the water. Supplementary data on this point were obtained in the laboratory by exposing snails to desiccation. The snails were separated from the soil by washing it through a series of screens. This method also gave data on the depth to which the snails had entered the soil at the time the pools dried.

Natural infection of the snails was determined (1), by placing in large battery jars specimens collected from pastures where infected cattle grazed to determine whether they were shedding cercariae, and (2), by crushing specimens and making microscopic examination for the intramolluscan stages.

CHARACTERISTICS OF THE GULF COAST PRAIRIE OF TEXAS

The Gulf coast prairie of Texas is 20 to 80 miles in width and, according to Carter (*3*), extends from the eastern border of the State "to the vicinity of the San Antonio River" in the west. It borders the Gulf of Mexico in the south, where it rises gradually from sea level to an elevation of about 100 feet in the northern part.

Geologically, this region is characterized by flat terrain consisting of heavy, clay soils covered with an abundant growth of coarse grasses and having poor drainage. It is traversed by a number of rivers, the broad flood plains of which form strips of red alluvial soil that constitute the rich, wooded bottom lands. According to Carter, the black prairie soil of the Lake Charles series is the most extensive type in the region. This soil is generally deep and rests on heavy, impervious clay subsoil. Numerous shallow depressions of natural origin known as hog wallows are characteristic of the black prairie soil.

As shown in figure 1, the climate of the Gulf coast prairie is mild, the average annual temperature being 68° to 70° F. The winters, which constitute the principal wet season, are both mild and brief, the periods of freezing temperature being few and of short duration. The summers, on the other hand, are long and hot and frequently have prolonged periods of little or no rainfall over large sections. The average annual rainfall is about 50 inches. The greatest precipitation generally occurs during July, September, and December and the lowest during January, February, March, and April (fig. 1). As shown also in figure 1, the average annual evaporation exceeds the average annual precipitation from the beginning of March until late in August, during which time the surface pools are usually dry. This period of dryness commonly extends into October. Rain occurs most frequently during midwinter and midsummer. The average relative humidity is about 80 percent.

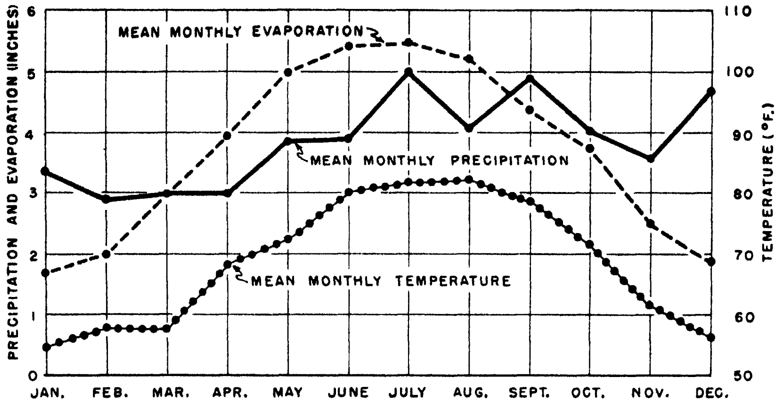


Figure 1.—Mean monthly precipitation, evaporation, and temperature in the Gulf coast prairie of Texas for the period 1914–41, as recorded at the Texas Agricultural Experiment Station, Substation 3, Angleton, Tex.

TYPES OF SNAIL HABITATS AND THEIR PHYSICAL CHARACTERISTICS

The habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie are of two types—semipermanent ponds and temporary pools.

The semipermanent ponds retain water well into the summer and often throughout the year. They are represented by roadside excavations, sloughs (fig. 2, *A*), and well overflows. Because of the open water on one side and the dry bank on the other, both of which serve as barriers to the snails, the habitable region of the semipermanent pond is confined to the narrow margin of shallow water and the wet mud on the bank.

The temporary pools contain water only when precipitation exceeds evaporation sufficiently to produce an accumulation of surface water. The types of pools in this group are of both natural and artificial origin. Natural depressions include marshy areas, shallow drainage courses, and numerous other low places known as hog wallows (fig. 2, *B*), in which water collects. Artificial pools arise from any land use that leaves an uneven surface, the principal causes being excavations, worn pasture roads, cowpaths, hoofprints, wagon or implement tracks (fig. 2, *C*), and cultivated fields that are permitted to revert to pasture without being properly leveled. The temporary pools, with their sparse vegetation and extensive soil-water peripheries, provide a much more favorable environment for snails than do the semipermanent pools. This fact is demonstrated by the data in table 1. Semipermanent pools included in a study made in Brazoria County, Tex., during essentially the same period and on similar types of soil had a much higher percentage of juvenile snails than did the temporary pools, indicating that a large proportion of the snails had died before reaching maturity.

Marshy areas resulting from overflows of wells have the shallow water and exposed mud of the temporary pools and the duration of the semipermanent ponds.

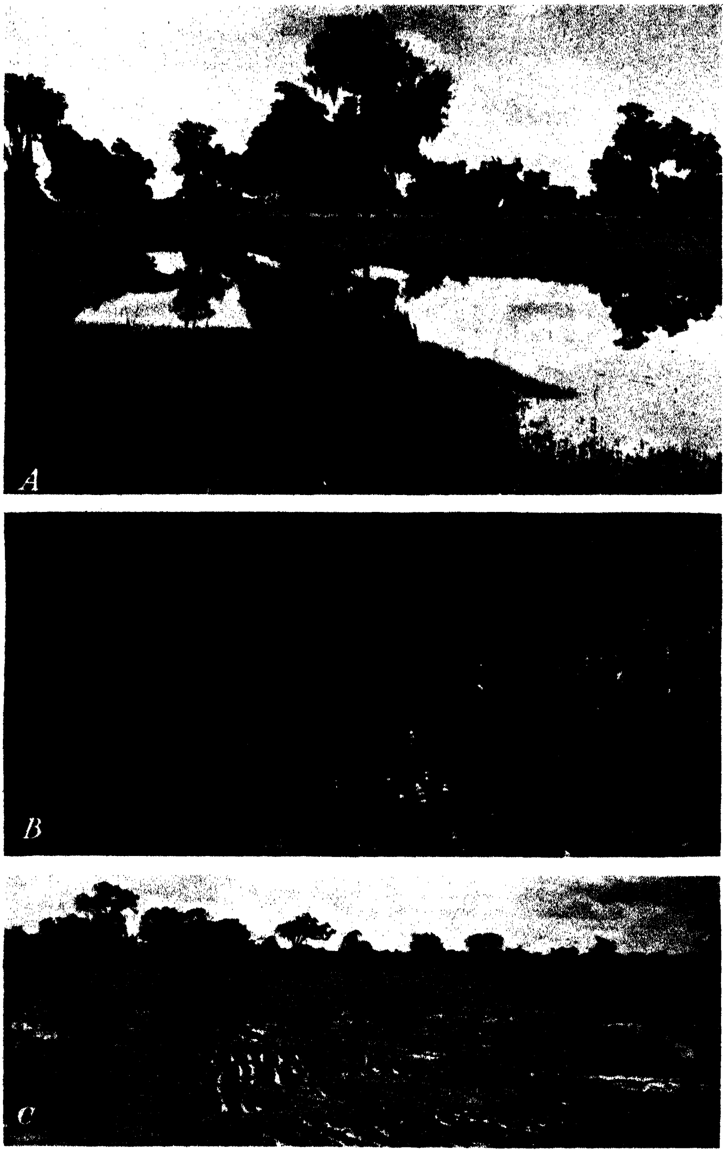


FIGURE 2.—Types of habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas: *A*, Slough of semipermanent type in which the narrow, flat banks (lower right) support large numbers of snails, whereas the steep banks (upper right) are without snails. *B*, Shallow surface depressions known as hog wallows, indicated in the photograph by the numerous areas of darker vegetation, constitute a large portion of the area. *C*, Wagon tracks and other depressions in this bottom-land pasture increase its danger as a snail habitat during the wet season.

TABLE 1.—*Proportions of juvenile and adult* Stagnicola bulimoides techella *in semipermanent and temporary pools in Brazoria County, Tex.*

Semipermanent pools				Temporary pools			
Date of collection	Snails collected	Proportion of—		Date of collection	Snails collected	Proportion of—	
		Juvenile snails	Adult snails			Juvenile snails	Adult snails
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
1941:				1941:			
July 15	221	62.7	37.3	July 30	163	28.8	71.2
July 31	550	69.3	30.7	Aug. 14	360	4.4	95.6
Aug. 18	400	57.8	42.2	Aug. 19	500	42.0	58.0
Sept. 17	720	98.5	1.5	Sept. 26	220	10.9	89.1
Sept. 26	336	83.6	16.4	Sept. 29	320	41.8	58.2

The two types of habitats have many physical similarities, particularly in type of soil and source of water. The pH of the water in the area studied ranged from 7.1 in certain well overflows to 8.4 in some of the temporary pools on alluvial soils. In general, temporary and semipermanent pools originating from rainwater were less alkaline on the black prairie soils than on the red alluvial soils. The hydrogen-ion content of the water appeared to have no influence on the distribution of the snails.

Temperatures in and around pools fluctuate greatly, depending on the season, time of day, amount and density of shade, and degree of exposure. Variations in temperature resulting from the last-mentioned factor are shown in table 2. The table also shows that during the summer months exceedingly high daytime temperatures prevailed in the shallow water and on the soil, whether wet or dry.

TABLE 2.—*Summer temperatures recorded in 2 types of habitats of Stagnicola bulimoides techella, in southern Texas under conditions of both drought and moisture*

Type of habitat	Date	Temperature of—			
		Air	Shallow water	Saturated mud at water's edge	Dry soil
		°*F.*	°*F.*	°*F.*	°*F.*
Low; well protected	June 26, 1940	91.4			146.3
	July 7, 1941	89.6	100.4	102.2	102.5
Flat; fully exposed	July 18, 1941	93.2	102.2	105.8	117.5
	Sept. 26, 1940	77			100.4

LIFE HISTORY

Egg masses of *Stagnicola bulimoides techella* are deposited most frequently on the wet mud at the water's edge or in the shallow water along the margin of the pool. In aquaria, egg masses have appeared more often on the exposed wet mud than in the water. Their size varies greatly, ranging from 2.5 to 12 mm. long by 1.8 to 4.2 mm. wide. The number of eggs counted in 36 masses ranged from 9 to 50 each with a mean of 22 eggs per mass.

Hatching of the eggs may occur as soon as 6 days after oviposition during the summer when the temperature is high. The young snails are about 0.6 mm. long when hatched and live on the saturated mud at the water's edge (fig. 3). Of the large numbers of eggs incubated in the laboratory, relatively few failed to hatch.

Growth of the young snails is very rapid when nutrition and moisture are favorable. Sexual maturity, which occurs when the shell reaches a length of about 4.5 mm., is attained as early as 14 days after hatching. Reproduction begins as soon as the snails reach sexual maturity and may continue 3 to 7 months, depending on environmental conditions.

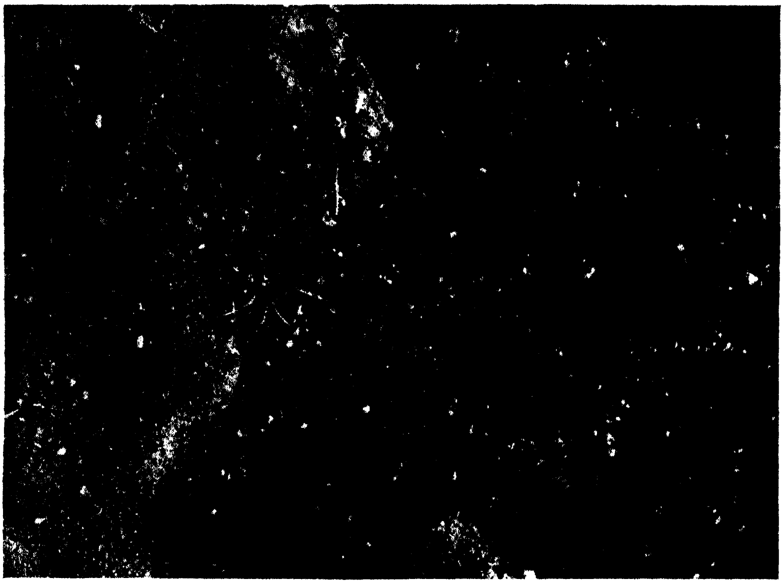


FIGURE 3.—Juvenile and adult snails on the wet mud and in the shallow water of a much-used and barren herd pen.

To determine the course of the life cycle of the snails in the field, including the number of generations and the time of the year when they occur, 102 collections in 40 localities, including more than 32,000 individuals, were made for a period of 2 years. The percentage of juveniles in the population for each month is shown in figure 4. As indicated by the figure, the June population is predominantly juvenile. Owing to the usual disappearance of the water from the habitats at this time, the young snails estivate in the soil during the period in which the pools are dry. Most of the old snails die without entering the soil. This fact was clearly demonstrated in a study of the snail population in one pool in southern Texas. A collection made on May 9, 1940, after the pool had dried, showed that most of the snails dead on the surface of the soil were adults (table 3). On October 30, after the water had reappeared in the pool, the surviving population was predominantly juveniles. Growth was rapid and

TABLE 3.—*Percentage of juvenile and adult Stagnicola bulimoides techella in the population (both living and dead) shortly before and immediately after estivation, which extended from May 9 to October 30, 1940, in a pool in southern Texas*

Date (1940)	Snails collected	Juveniles	Adults
	Number	*Percent*	*Percent*
Apr. 11	226	57.5	42.5
May 9	38	8.0	92.0
Oct. 30	942	98.4	1.6
Nov. 14	398	37.5	62.5

the population quickly passed from one of juveniles to one of adults, as shown by the percentages of each on November 14.

As indicated by the data in figure 4, obtained from extensive studies, mass oviposition takes place by the first of the year and the adult snails disappear for the most part. Thus, the longevity of the spring generation is about 6 months. The January population consists of a new generation of juvenile snails that originated from the June generation. These snails develop rapidly, oviposit, predominate in the population for a short time as adults, and then die, having lived about 6 months. Thus, under field conditions the general trend is

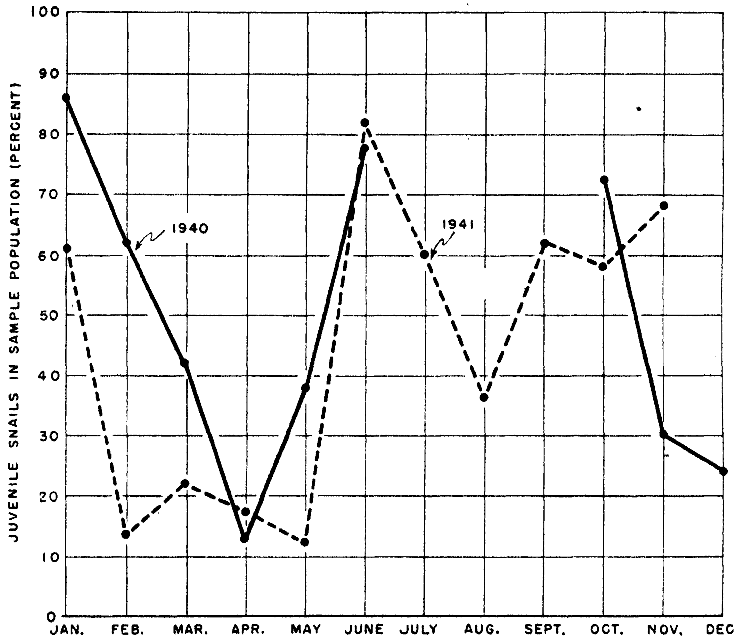


FIGURE 4.—Seasonal distribution of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas in 1940 and 1941. Pools were dry during July, August, and September 1940.

two distinct generations annually with some overlapping of reproduction. This condition exists whether the years be dry, as in 1940 when the pools were without water from June until the last of October, or very wet, as in 1941 when water persisted in them throughout the summer.

That the snails are capable of living much longer than the usual 6 months is shown by the fact that one individual survived in the laboratory for 25.5 months. This specimen was kept in a battery jar containing about 3 inches of mud to which water was added once monthly and permitted to remain 2 to 7 days, when it was drained off. The jar was kept in an open shed or in the laboratory, where it was protected from direct sunlight. When the soil began to dry, the snail burrowed into the mud where it remained until water was added. Both growth and reproduction occurred to a limited degree under these conditions.

EGG PRODUCTION

Since *Stagnicola bulimoides techella* is hermaphroditic, the entire population is capable of producing fertile eggs. This fact, together with the short juvenile stage and the long period of fecundity, accounts for the occurrence of a large population of snails in a habitat in a remarkably short time when the physical and nutritional conditions of the environment are favorable (fig. 3).

In the present studies, as shown in table 4, four laboratory-raised *S. bulimoides techella* began ovipositing 14 to 17 days after hatching and continued for 84 to 211 days, the average production being 5,112 eggs. Individual production ranged from 3,274 in 94 days to 6,479 in 129 days of laying time. The number of eggs was relatively low at first, rose to the maximum, and then decreased. A shortage of food in the aquaria resulted in a decrease in the number of eggs. The addition of food was reflected in a return to the previous number. Under favorable circumstances, the period of active oviposition may be as long as 7 months.

TABLE 4.—*Egg-production data for 4 laboratory-reared Stagnicola bulimoides techella, hatched May 30, 1940*

Snail No.	Date of beginning of oviposition	Number of eggs deposited during—							Total eggs	Date of death	Age	Period of oviposition	Mean daily eggs	Total egg masses	Mean daily egg masses	Mean eggs per mass
		June	July	August	September	October	November	December								
									Number		*Days*	*Days*	*Number*	*Number*	*Number*	*Number*
1	June 17	274	1,295	1,056	2,942	912	----	----	6,479	Oct. 31	152	129	50.2	303	2.3	21.5
3	June 15	147	850	1,561	716	----	----	----	3,274	Sept. 17	109	94	34.8	202	2.1	16.3
4	June 14	176	955	1,424	1,917	288	545	461	5,766	Jan. 10	226	211	27.3	414	2.0	13.9
6	June 17	214	2,308	2,099	309	----	----	----	4,930	Sept. 9	100	84	58.7	258	3.1	19.1
Total or average [1]		811	5,408	6,140	5,884	1,200	545	461	20,449			518	39.5	1,177	2.3	17.4

[1] Weighted average.

EFFECT, ON SURVIVAL, OF DESICCATION AND UNFAVORABLE TEMPERATURES

Since temporary pools are the principal habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas, drought constitutes the most important single factor operating against these snails. Whenever evaporation exceeds precipitation sufficiently long, as it normally does during the summer (fig. 1), the shallow, temporary pools slowly become dry, leaving the snails exposed to desiccation. No inclination is shown by the snails to follow the receding water, but protection against drying is sought by burrowing into the wet mud where they remain until the pool is restored by rains.

To determine the effect of drought on the survival of the snails, collections were made on June 26 and September 27, 1940—about 2 and 5 months, respectively, after the pool had gone dry—from a habitat where a large population had existed. The snails were collected from two adjacent plots, each 1 m. square and approximately 6 cm. deep. The soil was removed in definite layers, kept separate, and washed through a series of fine screens to determine the vertical distribution of the snails and their survival. The results are shown in table 5. The numbers of specimens lying on the surface and in the first centimeter of soil were about equal in both plots. Those lying on the top were dead, whereas more than 10 percent of those found in the loose, upper soil were alive despite its dryness and high temperature. Although fewer snails were found in the lower strata, more of them were alive. The lethal effects of drought in the habitat appear to be immediate rather than cumulative, since the number of snails surviving in the soil at the end of the summer was about equal to that present shortly after the pool had dried. The data show, therefore, that the snails, having once entered the heavy, clay soil,

TABLE 5.—*Vertical distribution and survival of Stagnicola bulimoides techella in the soil of a natural habitat during drought*

PLOT 1.—COLLECTIONS MADE JUNE 26, 1940 [1]

Soil stratum No.	Thickness of stratum	Temperature	Snails		
			Present	Alive	
	Centimeters	°*F.*	*Number*	*Number*	*Percent*
1	[2] 0	146.3	34	0	0
2	1.0	105.8	36	4	11.1
3	2.5	99.5	4	2	50
4	2.5	(3)	0	0	0
Total			74	6	8.1
PLOT 2.—COLLECTIONS MADE SEPTEMBER 27, 1940 [4]					
1	[2] 0	100.4	31	0	0
2	1.0	86	25	3	12.0
3	2.5	81.5	14	4	28.5
4	2.5	(3)	2	1	50.0
Total			72	8	11.1

[1] 60 days after pool had gone dry.
[2] Surface of soil.
[3] No record made, but temperature would be 2° to 3° lower than for stratum 3.
[4] 150 days after pool had gone dry.

are capable of withstanding at least 5 months of drought and summer temperatures of at least 105° F. However, the soil may be wet from time to time by sporadic summer rains, some of which may be very heavy.

Adult snails are capable of withstanding long periods of desiccation when protected from the direct rays of the sun. Specimens collected from pools in the field and kept in aquaria for 3 weeks were then placed in a dry, open dish in an unheated room, where they lived from March 19 to June 25, a period of 98 days. Another lot of large snails collected from the surface of drying soil, the orifices of which were filled with mud, was exposed to desiccation under the same conditions as those mentioned above. These snails lived from March 15 to August 20, a period of 158 days.

Although freezing temperatures occur in the Gulf coast prairie, they are of short duration and usually not severe, the lowest on record at the Texas Agricultural Experiment Station, Substation 3, Angleton, Tex., being 10° F. *S. bulimoides techella* continued to be active during this period, and no evidence of death was observed as a result of low temperatures. To determine further the ability of the snails to withstand low temperatures, adult specimens were put in a finger bowl containing water, placed in the ice box, and kept there for 33 days except for short periods, when they were taken out to ascertain whether they were alive. All the time that the snails were in the ice box, the water in the dish was covered with ice. No deaths occurred until the water in the dish was allowed to freeze solid, when most of the snails succumbed within a few hours; none survived more than a day.

Since the eggs of *S. bulimoides techella* are deposited on the moist mud at the water's edge or in shallow water, they are exposed to desiccation as soon as the pools begin to dry in the spring. That the eggs do not survive the summer drought is shown by the results of collections made in a pool immediately after a drought of 5 months' duration (table 3). Four days after the snails had emerged from the soil, 98.4 percent were juveniles. Of 942 snails collected, the smallest individuals were 1.5 mm. long, whereas the length of newly hatched snails is only 0.6 mm. Two weeks later, only 37.5 percent of the population were juveniles. Furthermore, the survival of eggs subjected to the lethal action of the sun's rays for 5 months and to soil temperatures of as high as 146.3° F., is unlikely.

Estivation is not limited to drought conditions only but may occur in pools containing water when the temperature becomes unfavorably high. In one semipermanent habitat where large numbers of snails were observed, none could be found 2 weeks later when the temperature of the saturated mud and shallow water reached 100.4° and 102.2° F., respectively. After a rain and lowered temperatures, the original population reappeared in its former proportion of adults and juveniles.

DISSEMINATION

No evidence of lateral migration by *Stagnicola bulimoides techella* has been obtained. As already stated, these snails do not follow the receding water in the habitats, and so accumulate in large numbers in the remnants of the pools. Lateral movements do occur, however, but they are passive on the part of the snails. Usually they take place during the runoff of high water resulting from the heavy and prolonged rainfall that frequently inundates the country. When the habitats are covered with deep water, the snails often rise to the top, where they cling inverted to the under side of the surface film of the water. The gentle flow of the water through natural drainage and the movements of wind disseminate them over wide areas.

The main cross-country drainage ditches constitute another means of disseminating the snails. Well overflows that empty into these drainage ditches form pools where the snails breed throughout the year. These places become the sources of infestation for pastures below and the drainage ditches become the avenues of distribution whenever sufficient rain occurs to produce a flow of water in them. Likewise, drainage ditches, the bottoms of which are improperly aligned, catch and retain water and form favorable habitats for the snails from which they may be carried downstream.

NATURAL INFECTION WITH THE LIVER FLUKE

Natural infection of *Stagnicola bulimoides techella* with *Fasciola hepatica* was found to occur throughout the year. Of 16,276 snails dissected during 1941 from pastures where infected cattle ranged, 83, or 0.0051 percent, were found to be infected. The highest rate of infection occurred in the fall and winter of 1941, which followed a very wet summer. The monthly rate of infection ranged from 0.07 percent in May to 6.2 percent in November. In one temporary pool where conditions for snail growth and parasitism were ideal, the rate of infection was 2.9 percent on October 28 and 6.6 percent 1 month later, whereas only 0.32 and 1.6 percent were infected on December 19 and January 14, 1942, respectively. No infection was found in snails in an adjacent semipermanent habitat (fig. 2, *A*) that harbored a large population of snails and that was accessible to the same cattle as the temporary pool. Sinitsin (*15*) reported 4 percent of *Stagnicola bulimoides techella* in the vicinity of Houston, Tex., to be infected with the liver fluke. He did not state, however, whether this involved the general population or a restricted one.

SUMMARY

Before effective measures against the liver fluke of cattle and sheep can be formulated, a knowledge of the bionomics of the lymnaeid snail that serves as its intermediate host is essential. A study of the literature shows that the bionomics of the various lymnaeid snails that serve as the intermediate hosts of the liver fluke throughout the world varies according to the species involved and the conditions under which they live.

The Gulf coast prairie of Texas, which is in the upper part of the Gulf coast region of that State, is a uniformly flat and low area with a mild, moist climate, and heavy, clay soils that are poorly drained. These conditions provide a favorable habitat for *Stagnicola bulimoides techella*, the common intermediate host of the liver fluke in the United States. Consequently, field and laboratory investigations in this area were made in 1940 and 1941 to study the principal types of habitats, the life history of the snails, their reproduction, dissemination, and related subjects.

The study showed that the snails live in the temporary and semipermanent pools that are scattered over the entire area. The pools are generally only slightly alkaline in reaction and have extensive soil-water peripheries where the snails live.

The life cycle of *S. bulimoides techella* follows a rather well-defined pattern with two generations annually. One generation occurs in midwinter and the other late in the spring. When the habitats become dry in the spring or early in the summer, the young snails estivate in the soil until the fall rains. Development of the snails is rapid under favorable conditions, being as short as 20 days from egg to egg. The adult snails usually die when about 6 months old, after the period of reproduction.

Under laboratory conditions, the snails were found to remain alive as long as 25.5 months, during which time standing water was present in the aquarium for 2 to 7 days only at the beginning of each month. The snails are capable of withstanding periods of desiccation as long as 5 months under field conditions when estivating in the soil or when exposed in an open dish in the laboratory. The lethal effect of drought on the snails when the pools become dry is immediate rather than cumulative.

Egg production of individual snails is high. They have been found to produce as many as 6,479 eggs during a life span of 5 months and a laying period of 129 days. The snails surviving the summer drought are predominantly juveniles; they emerge from estivation when the fall rains restore the pools, develop rapidly, and oviposit. The snails do not migrate, but they may be distributed widely by water during periods of heavy rainfall when the country is inundated.

The mean infection with liver fluke of 16,276 snails dissected during one year was 0.0051 percent. In one temporary pool the rate of infection in the latter part of November was 6.6 percent.

LITERATURE CITED

(1) ALICATA, J. E.
1938. OBSERVATIONS ON THE LIFE HISTORY OF FASCIOLA GIGANTICA, THE COMMON LIVER FLUKE OF CATTLE IN HAWAII, AND THE INTERMEDIATE HOST, FOSSARIA OLLULA. Hawaii Agr. Expt. Sta. Bul. 80, 22 pp., illus.

(2) ——— SWANSON, L. E., and GOO, G. W. H.
1940. METHODS OF CONTROLLING THE LIVER FLUKE OF CATTLE IN HAWAII. Hawaii Agr. Expt. Sta. Cir. 15, 23 pp., illus.

(3) CARTER. W. T.
1931. THE SOILS OF TEXAS. Tex. Agr. Expt. Sta. Bul. 431, 192 pp., illus.

(4) FRANCIS, M.
1891. LIVER FLUKES: THE COMMON FLUKE (DISTOMUM HEPATICUM). A NEW SPECIES (DISTOMUM TEXANICUM). Tex. Agr. Expt. Sta. Bul. 18, pp. 123–136, illus.

(5) HOFF, C. C.
1936. STUDIES ON THE LYMNAEID SNAIL, FOSSARIA PARVA (LEA). PART I: WINTER HABITS. Ill. State Acad. Sci. Trans. 29: 259–262.

(6) ———
1937. STUDIES ON THE LYMNAEID SNAIL, FOSSARIA PARVA (LEA). PART II: SEASONAL LIFE HISTORY. Ill. State Acad. Sci. Trans. 30: 303–306, illus.

(7) JESUS, Z. DE.
1938. THE DESTRUCTION OF LYMNAEA PHILIPPINENSIS FOR THE CONTROL AND ERADICATION OF FASCIOLIASIS IN RUMINANTS. Philippine Jour. Anim. Indus. 5: 581–595, illus.

(8) Jesus, Z. De, and Mallari, A. I.
1937. BIOLOGY OF LYMNAEA PHILIPPINENSIS, AN INTERMEDIATE HOST OF FASCIOLA HEPATICA AND FASCIOLA GIGANTICA IN THE PHILIPPINES. Philippine Jour. Anim. Indus. 4: 501–513, illus.
(9) Mehl, S.
[1932.] DIE LEBENSBEDINGUNGEN DER LEBEREGELSCHNECKE (GALBA TRUNCATULA MÜLLER). UNTERSUCHUNGEN ÜBER SCHALE, VERBREITUNG, LEBENSGESCHICHTE, NATÜRLICHE FEINDE UND BEKÄMPFUNGSMÖGLICHKEITEN. ARB. AUS. DER BAYER. Landesanst. f. Pflanzenbau u. Pflanzenschutz. Heft 10, 177 pp., illus.
(10) Peters, B. G.
1938. HABITATS OF LIMNAEA TRUNCATULA IN ENGLAND AND WALES DURING DRY SEASONS. Jour. Helminthol. 16: 213–260, illus.
(11) Ross, I. C. and McKay, A. C.
1929. THE BIONOMICS OF FASCIOLA HEPATICA IN NEW SOUTH WALES AND OF THE INTERMEDIATE HOST LIMNEA BRAZIERI (SMITH). Austral. Council Sci. and Indus. Res. Bul. 43, 62 pp., illus.
(12) Shaw, J. N., and Simms, B. T.
1929. GALBA BULIMOIDES LEA AN INTERMEDIATE HOST OF FASCIOLA HEPATICA IN OREGON. Science 69: 357.
(13) ——— and Simms, B. T.
1930. STUDIES IN FASCIOLIASIS IN OREGON SHEEP AND GOATS. Oreg. Agr. Expt. Sta. Bul. 266, 24 pp., illus.
(14) [Sinitsin, D. F.]
1929. [REDIAE AND CERCARIAE OF FASCIOLA HEPATICA FOUND IN WATER SNAILS, GALBA BULIMOIDES TECHELLA.] (Abstract) Jour. Parasitol. 15: 222.
(15) ———.
1933. STUDIEN ÜBER DIE PHYLOGENIE DER TREMATODEN. VI. THE LIFE HISTORIES OF SOME AMERICAN LIVER FLUKES. Ztschr. f. Parasitenk. 6: 170–191, illus.
(16) Swales, W. E.
1935. THE LIFE CYCLE OF FASCIOLOIDES MAGNA (BASSI, 1875), THE LARGE LIVER FLUKE OF RUMINANTS, IN CANADA, WITH OBSERVATIONS ON THE BIONOMICS OF THE LARVAL STAGES AND THE INTERMEDIATE HOSTS, PATHOLOGY OF FASCIOLOIDIASIS MAGNA, AND CONTROL MEASURES. Canad. Jour. Res. 12: 177–215, illus.
(17) United States Bureau of Animal Industry.
1940. REPORT OF THE CHIEF OF THE BUREAU OF ANIMAL INDUSTRY, 1940. 90 pp.
(18) ———
1941. REPORT OF THE CHIEF OF THE BUREAU OF ANIMAL INDUSTRY, 1941. 92 pp.
(19) Van Cleave, H. J.
1933. AN AMPHIBIOUS HABITAT ON VERTICAL SANDSTONE CLIFFS. Ecology 14: 149–151.
(20) ———
1935. THE SEASONAL LIFE HISTORY OF AN AMPHIBIOUS SNAIL, FOSSARIA MODICELLA, LIVING ON SANDSTONE CLIFFS. Ecology 16: 101–108, illus.
(21) Van Volkenberg, H. L.
1929. REPORT OF THE PARASITOLOGIST. Porto Rico Agr. Expt. Sta. Rpt. (1928): 36–38.
(22) ———
1934. PARASITES AND PARASITIC DISEASES OF CATTLE IN PUERTO RICO. Puerto Rico Agr. Expt. Sta. Bul. 36, 26 pp., illus.
(23) Walton, C. L.
1918. LIVER ROT OF SHEEP, AND BIONOMICS OF LIMNAEA TRUNCATULA IN THE ABERYSTWYTH AREA. Parasitology 10: 232–266, illus.
(24) ——— and Wright, W. R.
1926. HYDROGEN-ION CONCENTRATION AND THE DISTRIBUTION OF LIMNAEA TRUNCATULA AND L. PEREGRA, WITH A NOTE BEARING ON MOSQUITOES. Parasitology 18: 363–367.

A STUDY OF DORMANCY AND GERMINATION OF SEEDS OF CERCIS CANADENSIS[1]

By Michel Afanasiev

Associate horticulturist Oklahoma Agricultural Experiment Station

ANATOMY OF THE SEED

The seed of redbud (*Cercis canadensis* L.) is of the albuminous type. It has a very thin but strong seed coat, a considerable amount of perisperm and endosperm tissue, and, at the time of maturity, a well-developed embryo (fig. 1).

The seed coat (*a*), ranging in color from light tan to dark brown, is composed of small, thick-walled cells impermeable to water. These cells are cylindrical in form with the long axis perpendicular to the surface of the seed, and are commonly referred to as Malpighian cells. The perisperm (*b*), found immediately under the seed coat, is composed of fairly small, thick-walled cells. Unlike those of the endosperm, these cells are arranged in more or less regular rows, both radially and tangentially. The endosperm cells are thin-walled and irregular in shape and arrangement (*c*). The presence of a distinct perisperm in tree seeds is rather unusual, this tissue being normally either entirely absent or so similar in structure to the endosperm that it can hardly be differentiated from it. The mature embryo (*d*), occupying the central cavity of the endosperm, is about 4 mm. long with cotyledons 2½ to 3 mm. wide. In a ripe seed it is fully developed, and all its parts are completely differentiated. The seed is oval in form, somewhat flattened, and is borne in a pod varying in length from 6 to 8 cm.

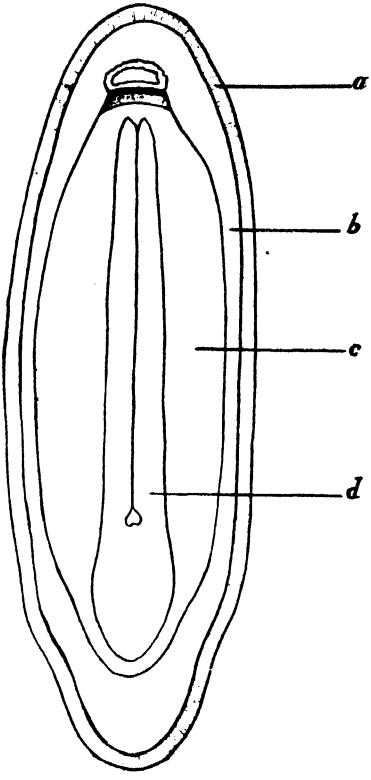


Figure 1.—Longitudinal section of redbud seed: *a*, Seed coat; *b*, perisperm; *c*, endosperm; *d*, embryo.

In Oklahoma the seed ripens at the end of August or the beginning of September, but remains on the tree throughout the winter. While still on the tree the seeds are subject to injury and destruction by

---

[1] Received for publication May 25, 1943. The work covered in this paper is part of an investigation carried on during the previous 4 years. (Bankhead-Jones research project) at the Oklahoma Agricultural Experiment Station, at Stillwater, Okla.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 10
Nov. 15, 1944
Key No. Okla.-31

616826—44——2

weevils. When collected in the late fall or winter the seeds were invariably worthless, being heavily infested with insects. In nature the seeds which escape injury and fall to the ground usually remain dormant for several years.

MATERIALS AND METHODS

Seeds of the redbud used in this investigation were either collected in Oklahoma (lots 39–0, 39–B, 39–C, 40, 41), mostly in the vicinity of Stillwater, or purchased from a commercial seed dealer in Boston, Mass. (38–A, 38–B). A few experiments, chiefly for the verification of the original results, were performed with seeds collected in Kansas (39–K). With the exception of a few small lots collected between June 28 and August 25 of 1939 and used for the specific purpose of determining some properties of immature seeds, all seeds were collected when completely ripe.

Seeds after being collected and air-dried for several days were stored in glass or metal containers, usually at a temperature ranging between 35° and 50° F. Germination tests, as a rule, were carried out on moist cotton in Petri dishes. However, in some instances indicated in the text, the seeds were tested also in soil, either in flats or in the nursery seedbeds; and in a few cases, tests were made in Erlenmeyer flasks as described elsewhere in this paper. Only sound seeds were used in all tests. These were easily separated from the decayed, empty, or otherwise poor seeds after a treatment with concentrated sulphuric acid followed by washing and soaking seeds in cold water. Poor seeds failed to absorb water in large quantities and usually remained thin and shriveled, while sound seeds imbibed water freely and swelled to several times their original volume in 8 to 12 hours.

The stratification medium used throughout the investigation was granulated peat moss purchased from a chemical company in Kansas City, Mo. When storage at low temperatures was needed, the samples were held either at 37° to 45° F. in an electric refrigerator or in a cold room in which the temperature varied irregularly from 35° to 53° F. with an average of 44°.

It is estimated that more than 50,000 seeds of redbud were used during the investigation.

CAUSES OF DORMANCY AND SEED TREATMENTS

CAUSES OF DORMANCY

Two factors are responsible for delayed germination of redbud seed. One is restriction of the intake of water caused by the nature of the seed coat, a characteristic very common in seeds of legumes (*6*, *9*).[2] Another lies in the embryo, which fails to grow even when the seed absorbs a large quantity of water. Several samples of sound fully swollen redbud seeds remained under germinative conditions in the writer's laboratory for as long as 716 days without germinating. Dormancy of the embryo is responsible for the failure of fully swollen seed to germinate. Excised embryos kept under conditions favoring growth usually exhibited phototropism and geotropism, produced chlorophyll, but failed to grow and produce seedlings. Only in a few instances have excised embryos been observed to grow to a considerable size (*2*). From the practical point of view, redbud embryos should be considered dormant and the seed treated accordingly.

---

[2] Italic numbers in parentheses refer to Literature Cited, p. 420.

SEED TREATMENTS

Two separate problems are involved in forcing the germination of redbud seed: (1) Modification of the seed coat in order to permit absorption of moisture which is necessary for afterripening; and (2) afterripening of the seed. The treatment of redbud seeds prior to germination does not differ from that of other seeds having similar causes of delayed germination.

The impermeable character of the seed coat can be modified by any of the three methods commonly used on the so-called "hard" seeds in commercial and experimental work (*8*, *12*, *13*). The standard treatment of seeds with concentrated sulfuric acid (sp. gr. 1,842) proved to be particularly effective, and once the requirement for any one lot had been determined, acid treatment was used throughout the period of investigation whenever the seed coats had to be made permeable to water. As would be expected, individual lots of seeds varied somewhat in the intensity of acid treatment required. No attempt was made to standardize the temperature at which the treatments were carried out. The temperature varied between 70° and 85° F.

At the beginning of the investigation, the optimum period of acid treatment was determined by germination tests of seeds treated for various periods. Later, however, the ability of treated seed to absorb water and the freedom from injury of treated seed were used as the criteria of the proper length of the treatment. Undertreated seeds when placed in water do not absorb it and their volume remains unchanged. Overtreated seeds, on the other hand, absorb water freely and swell to several times their original volume, but also exhibit definite sign of overtreatment in the form of "burned off," light-colored spots appearing on the seed coat (fig. 2). Correctly treated seeds when placed in water swell markedly in 8 to 12 hours, yet their coats remain smooth and intact. Owing to a certain degree of structural variation in seed coat of the same species, it is impossible to determine a "perfect" treatment for any lot of seeds. Any treatment that will render every seed permeable to water will result in overtreating at least a few of the seeds. Table 1 shows the proportions of swollen and "spotted" (injured) seeds caused by treatment with concentrated sulfuric acid followed by soaking in water.

TABLE 1.—*Proportion of swollen and injured seeds caused by acid treatment of various lengths of time; lot 39–K*

Condition of seeds	Not treated	Acid-treated for—				
		10 minutes	20 minutes	30 minutes	40 minutes	60 minutes
Percent of seeds swollen	0	5	60	100	100	100
Percent of seeds "spotted"	0	5	15	35	85	90

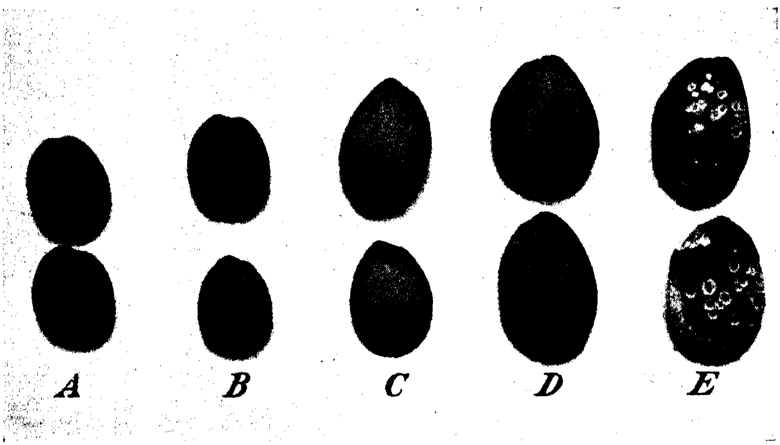


FIGURE 2.—Redbud seeds after various periods of sulfuric acid treatment followed by soaking in cold water for 24 hours (lot 39–O): *A*, Neither treated nor soaked; *B*, not treated but soaked in water; *C*, acid-treated 10 minutes; *D*, acid-treated 20 minutes; *E*, acid-treated 40 minutes.

In determining the optimum period of treatment the question arises as to whether it is more desirable to obtain permeability in all seeds and have some seeds overtreated or to leave part of the seeds with the seed coats still impermeable to water but avoid as much as possible the injury to the seed coats from overtreating. It should be stated that a slight injury to the seed coat does not affect the soundness of the kernel or interfere with the afterripening and germination of the seed, although more serious injury may pave the way for its decay and ultimate destruction. The shortest period of treatment that causes all seeds to absorb water does not usually result in a critical injury to any of the seed and should therefore be considered as the optimum.

The needed length of treatment as determined by the ability of seed to absorb water can be ascertained in a few hours. The optimum treatment of each lot used in this investigation was found by treating small lots of seed for from 10 to 60 minutes. Seeds less than 1 year old responded best to treatment for from 25 to 30 minutes. With the lengthening of the storage period between the time of collection and the time of treatment (age), the response of the seed to acid treatment became slower. The most effective treatment (30 minutes) during the first winter after collection was not sufficiently long when applied to the same lot of seed after a storage of 15 months, as is indicated by the accompanying tabulation:

Lot No.	*Optimum period of acid treatment—minutes*
38–B	30
39–K	25
39–O	30
38–B (after 15 months of dry storage)	45

Hot or boiling water was another medium that was effective in rendering the seed coats permeable to water. Only a small portion of the seeds of lot 38–B soaked in water heated to 180° F. were made permeable to water, whereas submerging them in boiling water for ¼ minute, ½ minute, 1 minute, and 2 minutes resulted in absorption of water by 62.5, 86.5, 97.5, and 97.5 percent of the seeds, respectively. Seeds of the same lot properly treated with concentrated sulfuric acid (30 minutes) responded 100 percent to the treatment (table 2).

TABLE 2.—*Comparative effectiveness of hot water and sulfuric acid treatments on permeability of seed coat to water, lot 36–B*

Treatment	Percent of seed swollen after 35 days in stratification	Percent germination after indicated days in stratification		
		7	21	35
Boiling water, ¼ minute	62.5	0.5	12.0	28.5
Boiling water, ½ minute	86.5	1.5	26.0	44.0
Boiling water, 1 minute	97.5	2.5	18.0	47.0
Boiling water, 2 minutes	97.5	4.5	6.5	4.5
H_2SO_4, 30 minutes	100.0	4.0	60.5	[1] 83.0
Hot water (180° F.)	Few	0	0	1.0

[1] Stratified 28 days.

Use of boiling water even for 1 or 2 minutes has never given as favorable germination results as did the treatment with concentrated sulfuric acid. Although boiling the seed for ½ minute or less did not sufficiently modify the seed coats in all seeds, boiling for 1 or 2 minutes damaged embryos in at least some of the seeds. Lengthening the period of boiling-water treatment from ¼ to ½ minute or to 1 minute increased the percentage of seeds rendered permeable to water as well as the final percentage of germination. Boiling the seed for 2 minutes reduced germination markedly. Attempts to modify the seed coat by ether, which was found to be effective in increasing the percentage of germination of seeds of *Robinia pseudoacacia* (*9*), proved to be unsuccessful with redbud. Mechanical scarification was highly effective on redbud when each individual seed was scratched, cracked, or sandpapered. No mass treatment of seeds by a mechanical scarifier was tried.

Since the impermeability of the seed coat to water is not the only cause of the delayed germination of redbud seed, modification of seed coat alone is not sufficient to break its dormancy completely: stratification of the seed is also necessary.

Many thousands of seeds of several crops were subjected to stratification during this investigation. The results of seven series of tests (typical for the redbud seed) are presented in table 3. The stratification requirements of individual lots varied considerably, as did those of individual seeds of any one lot. Yet despite these variations two definite conclusions concerning stratification requirements of redbud seeds appear to be justified.

(1) All seeds of redbud must complete afterripening and cannot be forced to germinate unless previously kept under conditions favoring this process. Stratification at 35° to 45° F. favors afterripening.

(2) A stratification period of 5 to 8 weeks at 35° to 45° F. is required for the completion of afterripening of 90 percent or more of seeds in practically all instances. In individual cases, germination of more than 80 percent was secured after less than 4 weeks of stratification (lots 38–B and 39–0) and in one lot (38–B) the seeds germinated to the extent of 94 percent after only 22 days of stratification.

TABLE 3.—*Effect of the length of the stratification period on afterripening of acid-treated*[1] *seeds of redbud*

Days in stratification	Percent germination in lots—[2]						
	38-A	38-B	38-B_1	38-B_2	39-K	39-O	39-C_1
0					0	0	0
5			4.0	0			
7					15.0	4.0	
8		28.0					
11			4.0	3.0			
14							10.0
15		50.0	4.0	3.0			
21					42.5	60.5	13.0
22		94.0					
25	40.0		10.0	17.0			
28					79.5	83.0	22.0
29	70.8	88.0					
30			14.0	11.0			
35	50.0	96.0	34.0	34.0	97.0		31.0
40			26.0	50.0			
42					99.0		80.0
46		92.0	55.0	89.0			
49							90.0
50			84.0	95.0			
55	47.0	81.0					
56	100.0	88.0			100.0	96.0	
60			88.0	95.0			
63					99.5	95.0	
64							97.0
65			53.0	72.0			
70			23.0	60.0	98.5	94.5	
75			16.0	45.0			
80							
81			56.0	86.0			
84							
86			57.0		99.0	97.0	
91					100.0	99.5	
92							
93			9.0	1.0			
98						100.0	
100				3.0			
105						99.5	

[1] Seeds treated 25 or 30 minutes according to the requirement of individual lots.

[2] The dates in 1939 on which first germination tests were started for the different lots follow: 38-A, Feb. 27 38-B, Mar. 15; 38-B_1, June 27: 38-B_2, June 27; 39-K, Nov. 10; 39-0, Nov. 10; 39-C_1, Oct. 4.

Extension of the stratification period for a few weeks beyond that needed for the completion of afterripening does not seem to interfere seriously with the ability of the seed to germinate. Therefore it appears safe to delay planting of afterripened seed should such delay become necessary. The only two samples of seeds in which germination was reduced with the extension of the stratification period were those stratified on June 22, 1939 (lots 38-B_1 and 38-B_2). Since germination of afterripened redbud seed is adversely affected by extremely high temperature (see table 7), it is very probable that this factor was responsible for the reduction in germination of seeds of these lots rather than the extension of stratification beyond the period 7 to 9 weeks, at which time germination reached its maximum. Germination tests of these two lots were carried on into the late summer when the temperature in the laboratory often exceeded 90° F., whereas the temperature of germination of other lots varied between 70° and 85°.

In addition to the sulfuric acid and stratification method of germinating redbud seeds, various other "forcing" methods and agents were tried. A summary of these treatments and of the results obtained is presented in table 4. For the purpose of comparison, seeds stratified after a treatment with sulfuric acid are also included in the table.

None of the treatments were so effective in afterripening of redbud seed as the combination of proper sulpuric acid treatment and stratification.

TABLE 4.—*Effect of various treatments on afterripening and germination of redbud seeds: lot 38–B*

Treatment	Highest germination	
	Percent	Period in days
None	0	716
H_2SO_4 30 minutes	10.0	365
Stratification 125 days	1.0	90
H_2SO_4 30 minutes and O_2 100 percent for 7 days	0	[1] 7
H_2SO_4 60 minutes, followed by freezing for 21 days	0	[2] 18
H_2SO_4 30 minutes, followed by stratification at weekly alternations of temperature 35°–75°	3.0	50
H_2SO 60 minutes, and concentrated HNO_3, 1 minute	25.0	24
H_2SO_4 15 minutes, followed by stratification for 36 days (41° F.)	46.0	18
H_2SO_4 30 minutes, followed by stratification for 35 days (41° F.)	96.0	8
H^2SO_4 60 minutes, followed by stratification for 36 days (50° F.)	29.0	39
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 formic acid for 20 hours	38.0	15
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 NaOH for 20 hours	56.0	15
Boiling water 2 minutes followed by stratification for 35 days (41° F.)	4.5	33

[1] In oxygen.
[2] All dead.

Substitution of freezing for stratification not only failed to bring about afterripening but actually injured the seed. Injury to the embryo caused by mistreatment, mishandling, or by application of certain chemicals always became evident in a few days after the seeds were placed under conditions favoring germination. The loss of viability of injured seeds manifested itself first in a rapid development of fungi on and around the seeds and then in a complete decay and destruction of the affected seeds.

Use of concentrated nitric acid to supplement sulfuric acid treatment caused germination of 25 percent of seeds without stratification but damaged the remainder of the lot. Application of weak solutions of formic acid and sodium hydroxide markedly hastened germination as compared with the application of H_2SO_4 alone, and increased the total percentage of germinated seeds. Germination of seeds not treated with sodium hydroxide was 29 percent as compared to 56 percent for seeds soaked in sodium hydroxide for 20 hours. Increasing the oxygen concentration of the air to 100 percent did not produce as good germination results as stratification, although it had a very pronounced stimulative effect on germination of afterripened seeds, as will be shown later.

An attempt was also made to hasten afteripening in stratification by holding acid-treated seeds in pure oxygen before placing them in stratification. Such treatment not only failed to bring about the desired result but actually lowered germination. After 6 weeks of stratification, 98 percent of seeds not treated with oxygen had germinated while seeds held for 24 hours in pure oxygen prior to stratification germinated to the extent of only 36.5 percent (table 5). A similar effect of the increased oxygen supply on afterripening has been observed in the case of red cedar seeds (*3*, *10*).

TABLE 5.—*Effect of vitamin B_1 and pure oxygen on afterripening*

Treatment	Percent germination after stratification for—		
	2 weeks	4 weeks	6 weeks
H_2SO_4, 30 minutes; vitamin B^1, 24 hours; stratification	18.0	38.5	99.5
H_2SO_4, 30 minutes; pure oxygen, 24 hours; stratification	2.0	6.5	36.5
H_2SO_4, 30 minutes; soaking in water, 24 hours; stratification	15.5	27.5	98.0

In view of frequent claims of the extraordinary stimulative effects of vitamin B_1 on plant growth, a few tests to obtain information as to its value were carried out. One of these dealt with the effect of the vitamin on afterripening. Seeds of lot 39–C, after being treated for 30 minutes with concentrated sulfuric acid, were soaked for 24 hours in a solution containing 4 mg. of vitamin B_1 in a liter of water. After the completion of the treatment the seeds were stratified and the rate and degree of afterripening were compared with those of seeds which were treated with sulfuric acid and then stratified without being soaked in the vitamin solution. In each test germination of vitamin-treated seeds was slightly higher than that of untreated seeds (table 5). The differences in total germination, however, were too small to be significant.

MICROCHEMICAL TESTS

Observations on stored food and three oxidizing enzymes in the seed were made by means of the following tests and reagents: Protein-biuret reaction, fats-Sudan IV; reducing sugars-Flückiger reaction; starch-iodine potassium iodide solution; oxidase-alcoholic solution of gum guaiac; peroxidase-alcoholic solution of gum guaiac and 3-percent solution of hydrogen peroxide; catalase-3-percent solution of hydrogen peroxide neutralized with calcium carbonate.

Afterripening of redbud seed is accompanied by or consists of a number of chemical and physical changes within the seed, more or less like those found in other seeds (*1, 3, 5, 10*). Microchemical tests made on dormant redbud seed revealed large quantities of protein and fats, both in the endosperm and the embryo. Protein was particularly abundant in the cotyledons. Fats were abundant in the tips of the cotyledons and in the endosperm, yet present also in the radicle and the hypocotyl. Neither starch nor sugars were detected in a dormant seed. Oxidase was absent or inactive but the presence of peroxidase and catalase [3] was easily detected.

The embryo of a dormant seed is slightly acid (pH 6.7). The moisture content of an air-dry dormant seed including the seed coat is equal to approximately 10–10.5 percent of the fresh weight of the seed.

The microchemical tests at various stages of afterripening and during germination were made on seeds after 2, 4, 6, and 8 weeks of stratification preceded by treatment with concentrated sulfuric acid. Further statements pertaining to the changes in the amounts of various substances in seeds are based on the changes in the intensity of the reactions brought about by the application of proper reagents. A summary of the changes resulting from afterripening and germination is presented in table 6.

---

[3] Changes in catalase activity were determined quantitatively and are discussed later.

TABLE 6.—*Summary of various changes within the redbud seed as a result of afterripening and germination*

[Differences in number of crosses indicate relative changes in the amount of individual substances]

State of seed	Proteins	Fats	Starch	Reducing sugars	Oxidase	Peroxidase	Catalase [2]	pH	Moisture content (percent fresh weight)
Dormant [1]	XXX	XX	None	None	X	None	11. 25	6. 7	51. 0
Stratified 2 weeks	XXX	XX	None	None	X	X	13. 5	......	55. 6
Stratified 4 weeks	XXX	X	None	Trace	X	X	17. 0	6. 3	55. 8
Stratified 6 weeks	XXX	X	None	Trace	X	X	18. 0	6. 2	57. 4
Seed germinated in stratification after 8 weeks	XX	X	XXX	X	XX	XX	23. 4	6. 5	63. 5

[1] After sulphuric acid treatment and 24 hours of soaking.

[2] Cubic centimeters of O_2 evolved from 5 cc. of H_2O_2 in 10 minutes per 0.1 gm. of dry material.

As afterripening progressed the amount of proteins and fats decreased markedly, particularly the latter. However, after 6 weeks of stratification there still was a fair quantity of both in the endosperm and much protein in the embryo. Fats were still present in cotyledons and in the radicle but had disappeared from the hypocotyl.

The presence of reducing sugars was detected in seeds after 4 weeks of stratification. At that time sugars appeared in the cotyledons and in the radicle but were absent from the endosperm. The first appearance of reducing sugars in the endosperm was noted after 6 weeks of stratification, and even then only in very small amounts in the area adjacent to the embryo.

After 2 weeks of stratification oxidase appeared in the cotyledons, along their inner edges. After 4 weeks it became active throughout both the endosperm and the embryo. Peroxidase and catalase increased continuously throughout the period of afterripening. The reaction of the kernel changed from the original pH of 6.7 in a dormant seed to 6.3, 6.2, and 6.5 after 4, 6, and 8 weeks, respectively, of stratification. As a result of sulfuric acid treatment and soaking overnight, the moisture content of the seed increased from 10.3 percent to 32.6 percent of the weight of fresh material. Further soaking of the seed at room temperature (for a total of 24 hours) raised the water content of the seed to 51.0 percent. During 6 weeks of stratification, moisture in the seed remained more or less constant, fluctuating between 54.3 and 57.4 percent.

GERMINATION

MICROCHEMICAL CHANGES

Germination is accompanied by a sharp increase in moisture content of the seed, further reduction in the amount of proteins and fats, increase in the amount of reducing sugars and in the activity of oxidase, peroxidase, and catalase, a slight reduction in the acidity of the embryo, and a sudden appearance of a large quantity of starch (table 6). Starch is abundant in the cotyledons, the outer part of the hypocotyl, and in the tip of the radicle, although some appears also in the endosperm.

TEMPERATURE

Germination of afterripened redbud seed takes place at a rather wide range of temperatures, from 33° to 100° F., and probably even higher. Tests to determine the range of germination temperature and the optimum temperature were conducted on a total of 3,200 seeds, one-half of which, after being treated with sulfuric acid for 30 minutes, were stratified at 41° F. for 6 weeks and the other half for 8 weeks. Table 7 presents a summary of the progress of germination of 16 samples of seed (200 each) placed and kept in germinators at various temperatures, from 33° to 100° F.

TABLE 7.—*Germination of redbud seeds as affected by temperature*

Germination temperature (° F.)	Percentage germinations [1] after—			
	2 days	4 days	6 days	8 days
33°	0	0	0.7	0.7
42°	.25	.25	1.7	1.7
48°	.50	3.75	15.5	24.0
60°	25.2	55.7	84.2	91.0
70°	72.2	91.2	96.5	96.5
80°	87.5	90.7	91.2	91.2
90°	90.5	92.0	93.0	93.0
100°	29.0	45.5	50.5	53.7

[1] Average of 2 samples, one stratified for 6 weeks, the other for 8 weeks.

Within the range of 33° to 90° F. the rate of germination was directly affected by the temperature. Two days after the seeds had been set in germinators, 90.5 percent germinated at 90° while germination at 48° and below was less than 1 percent. After 4 days, germination of more than 90 percent was obtained at 70°, 80°, and 90°, and after 8 days more than 90 percent of the seeds germinated also at 60°. At 100° germination rose from 29.0 percent in 2 days to 53.7 percent in 8 days. The total germination (in 8 days) was highest at 70° (96.5 percent), although at that temperature it proceeded more slowly than at 80° or 90°. The temperature of 70° appears to be the optimum for germination of redbud seed. It happens to be the most favorable temperature also for growth of small seedlings. At a temperature of 60° and below seedlings grew more slowly than at 70°, although they apparently remained normal and sound. At a temperature of 80° and above, a certain degree of deterioration was evident among the seedlings. This was particularly evident at 100° at which temperature many of the seedlings rotted and others assumed an unhealthy abnormal appearance.

OXYGEN

Although afterripened redbud seeds germinate freely and rapidly in the normal atmosphere, and even under water when air is bubbled through it, an increase in the oxygen content of the atmosphere or the addition of it to water had an exceptionally stimulative effect on germination. Whereas germination of well afterripened seeds under normal conditions may be expected to be completed in a period of

6 to 8 days, afterripened seeds kept in pure oxygen were observed to germinate to the extent of 100 percent in 2 days.

In the investigation of the effect of oxygen on germination, a sample of seeds was placed in a cheesecloth bag suspended in a flask filled with pure oxygen. A few cubic centimeters of water were left at the bottom of the flask to prevent drying of the seeds and to provide the necessary moisture for germination. Another sample was placed in 100 cc. of distilled water through which oxygen was bubbled at the rate of 20 bubbles per minute. Checks for these treatments were a standard germination set-up (Petri dishes with moist cotton) and a sample of seeds placed under water. The original intention was to keep the seeds under treatment for 2 days and then to transfer them to the normal conditions of germination for comparison of their behavior with that of normally handled seeds. However, at the end of the first day, 1.5 percent of the seeds serving as a check in a Petri dish had germinated, while germination of seeds held in pure oxygen was 71.5 percent. At this time the seeds were removed from the oxygen flask and placed in moist cotton in a Petri dish in normal atmosphere. One day after this transfer germination of the oxygen-treated seeds rose to 90 percent while that of untreated seeds was only 13.5 percent. In a similar experiment carried out prior to the one just discussed, afterripened seeds kept in pure oxygen for 48 hours germinated 100 percent during that period as compared with 18 percent germination of seeds held in the normal atmosphere (table 8).

Table 8.—*Germination of afterripened seed under the influence of variations in the oxygen content of air and water*

Treatment	Percentage germination [1] after—				
	1 day	2 days	3 days	4 days	5 days
48 hours in 100 percent oxygen	10	100.0			
Check (normal atmosphere)		18.0			
24 hours in 100 percent oxygen	71.5	90.0		96.5	97.5
Check (normal atmosphere)	1.5	13.5		90.5	94.0
Set in nonaerated water	0	0	0	0	0
Set in water with O_2 bubbling through		92.0			

[1] Days from the time of setting of the experiment.

Bubbling oxygen through water in which afterripened seeds were placed had nearly the same effect as keeping the seeds in pure oxygen. Under these conditions germination of 92.0 percent was secured in 2 days. No germination took place among the seeds kept continuously under nonaerated water.

VITAMIN B_1

While treatment of nonafterripened seed with vitamin B_1 might have had a slight stimulative effect on the process of afterripening, the vitamin applied to afterripened seed was found to be entirely ineffective in stimulating germination. Afterripened seeds treated for 24 hours with a vitamin B_1 solution of 4 mg. per liter germinated almost at the same rate and to the same extent (95.5 percent) as the seeds that were not treated (97.5 percent).

EFFECT OF DRYING ON AFTERRIPENED SEEDS

During a normal procedure of afterripening, planting, or other handling of seeds, there is always a chance for them to dry more or less. Accidental drying of afterripened seeds may in some instances reduce germinability, throw seeds into a state of secondary dormancy (*3*), or even destroy their value completely by reducing their viability (*7*). Knowledge of the effect of drying on afterripened seeds seems to be of practical importance, suggesting the degree of care to be exercised while handling, storing, or shipping them.

Sixteen hundred seeds treated 30 minutes with sulfuric acid and kept in stratification for 55 days were placed in the laboratory at 75° to 85° F. and allowed to dry. From time to time samples of the seeds were tested for their ability to germinate. The results, presented in table 9, show that drying for 6 days had no effect on the continued ability of an afterripened embryo to resume growth. After 10 days of drying, germination fell off from the original of 96 percent to 90 percent, but when dry storage was extended to 30 days germination was reduced to 78 percent. Further extension of the dry storage to 60 and 90 days lowered germination to 4 and 6 percent, respectively.

TABLE 9.—*Effect of drying of afterripened seeds*[1] *on their ability to germinate*

Percent of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)	Period of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)
0 (check)	96	56.4	10	90	10.2
1	98	6.2	30	78	11.7
2	94	6.9	60	4	11.3
4	92	7.7	90	6	
6	98	9.0			

[1] All seeds treated 30 minutes with concentrated sufluric acid, then stratified 55 days.

A sharp decline in the germination ability of afterripened seeds kept dry for 60 and 90 days was not caused by an additional loss of moisture Under conditions of dry storage (temperature 75° to 85° F.) moisture equilibrium in seeds was reached after 24 hours of drying. Continued exposure of seeds to a temperature of 75° to 85° beyond that period resulted in an increase in moisture content of the seed which was probably due to the increase in the humidity of the surrounding atmosphere.

Drying afterripened seeds in an oven affected the viability of the seeds in various degrees, depending not only on the temperature of drying but also on the moisture content of the seeds at the time when the seeds were placed in the oven. When partially dried, redbud seeds were able to withstand considerable heat. In this investigation, seeds dried at room temperature for 4 days with moisture content reduced to 7.7 percent of the total weight of the seeds lost in viability from the original of 96 percent to 81 percent after being exposed to a temperature of 167° for 2 days.

The ability of partially dried seeds to withstand abnormally high temperatures is not uncommon. The present technique of seed extraction of coniferous species involves the use of dry kilns in which the temperature is raised to 110° and 120° F. (*14*).

CATALASE ACTIVITY

Catalase determinations in redbud seed were made by the method described by Davis (4) and adopted since by many other workers for measuring the activity of this enzyme in plant tissues. During the present investigation the entire seeds rather than kernels were tested for catalase activity because it is extremely difficult, if at all possible, to separate the seed coat from the perisperm. The amounts of various substances used in each catalase test were as follows: Water, 12 cc.; 3-percent solution of hydrogen peroxide, 5 cc.; plant material (seeds), approximately 600 mg.; $CaCO_3$ to equal the weight of plant material; and a pinch of pure quartz sand. In the following discussion catalase activity is expressed in terms of the number of cubic centimeters of oxygen evolved from 5 cc. of hydrogen peroxide in 10 minutes per 0.1 gm. of dry material of the seeds.

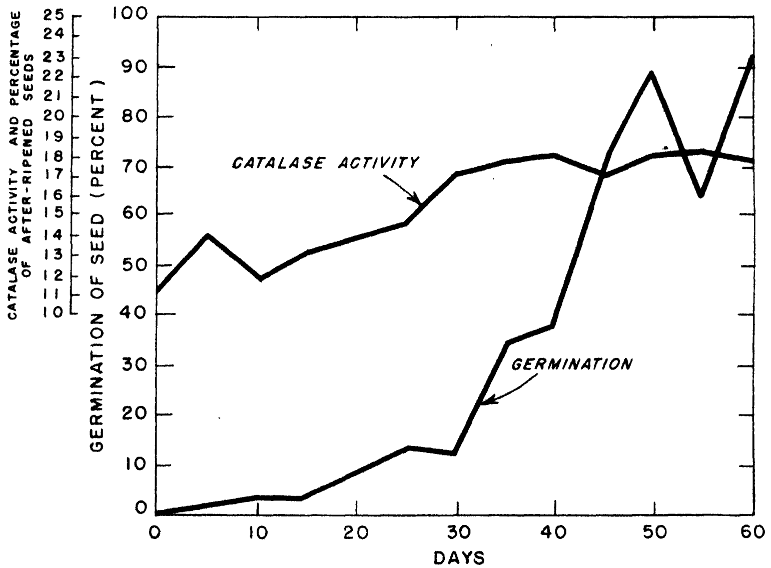


FIGURE 3.—Relation between degree of afterripening and catalase activity in redbud seed. Catalase activity is expressed in terms of cubic centimeters of oxygen given off in 10 minutes by 0.01 gm. of dry material.

Catalase activity was determined in dormant dry seeds, seeds treated with concentrated sulfuric acid and soaked overnight, and seeds kept in stratification for various periods of time under conditions favoring afterripening. To obtain a good representative sample of the entire lot, 75 seeds were used in each test. On the days on which the tests were run, samples of seeds were placed in germinators to determine the degree of afterripening and the readiness of the seeds to germinate. The results of these tests are given in table 10 and figure 3.

Catalase activity in stratified redbud seeds increased with afterripening from 11.25 in dormant seed to an average of 17.95 in seeds more than 90 percent of which had completed their afterripening.

TABLE 10.—*Catalase activity and afterripening during stratification* [1]; *lot 38–B*

Period of stratification (days)	Lot 38–B_2		Lot 38–B_1	
	Catalase [2]	Germination	Catalase [2]	Germination
0	11.25	0	11.25	0
5	13.26	0	14.85	4.0
11	11.20	3.0	12.35	4.0
15	13.50	3.0	13.00	4.0
20	12.80	--------	14.90	--------
25	15.00	17.0	14.30	10.0
30	17.00	11.0	17.30	14.0
35	18.05	34.0	17.55	34.0
40	18.00	50.0	18.36	26.0
46	17.50	89.0	16.70	55.0
50	18.60	95.0	17.65	84.0
55	18.85	81.0	17.50	47.0
60	18.25	95.0	17.65	88.0

[1] All seeds treated for 30 minutes with concentrated H^2SO^4.

[2] Catalase activity expressed in terms of the number of cubic centimeters of oxygen evolved in 10 minutes by 0.1 gm. of dry material.

The increase was very gradual, not entirely uniform, and not strongly correlated with the increase in germination. This suggests one of two possibilities: either catalase activity is more or less independent of the progress of afterripening, its intensity being simply controlled by the same external factors and in the same direction (though at different rates) as the afterripening; or, if changes of catalase activity are a part of the afterripening, they precede some other changes that are necessary for the completion of afterripening. The writer is inclined to consider the changes in catalase activity as a part of afterripening because of the close interrelationship between this and other processes on which the progress and the completion of the afterripening depend. Whether the relationship between catalase activity and the progress of other phases of afterripening is constant under all conditions and for all lots of seeds is, of course, open to question. Considerably more work is needed along this line.

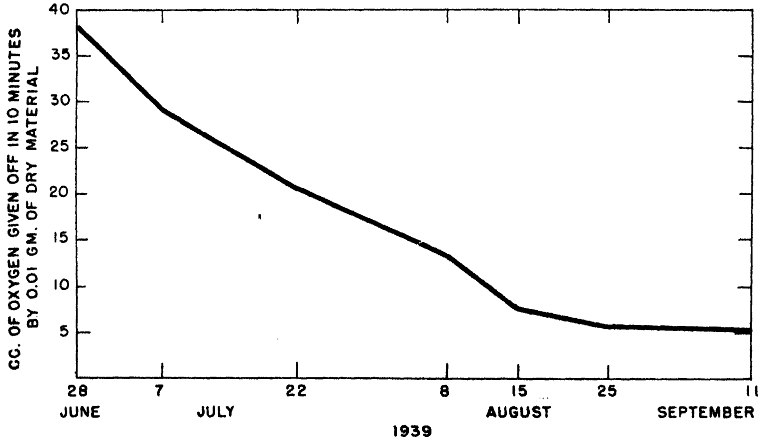


FIGURE 4.—Catalase activity as affected by the maturity of redbud seed. At the time of the two last tests (August 25 and September 11) the seeds were ripe.

In the course of the investigation catalase activity during the ripening process was also studied. Samples of seeds from a single plant were collected and catalase determinations made on various dates from June 28, 1939, to September 11 of the same year. On the former date the seeds were small, green, and soft. The two last tests (August 25 and September 11) were made on seeds which appeared to be completely ripe. The assumption that the seeds were ripe during the last two tests is substantiated by the results of catalase determinations, which showed that catalase activity attained a certain degree of stability.

As was expected (*11*), catalase activity fell off sharply with the ripening of the seed. At the time the seed reached maturity catalase activity was less than one-seventh of what it was 2 months earlier (fig. 4).

SUMMARY AND CONCLUSIONS

Delayed germination of seed of redbud (*Cercis canadensis* L.) is caused by the impermeability of the seed coat to water and by the dormancy of the embryo.

The seed coat can be rendered permeable to water by any of the standard treatments used with "hard-coated" seeds; namely, soaking of seed in concentrated sulfuric acid, hot water or boiling water bath, and mechanical scarification. The optimum treatment with sulfuric acid was of 30 to 35 minutes' duration

Extension of the storage of ripe dormant (unstratified) seeds to 15 months resulted in the lengthening of the required period of acid treatment from 30 to 45 minutes.

Stratification of acid-treated seed at low temperature (35° to 45° F.) was found to be effective in afterripening. The average period of stratification causing afterripening of not less than 90 percent of seeds varied between 5 and 8 weeks.

Holding afterripened seed in pure oxygen for 24 or 48 hours resulted in a marked increase in the rate of germination.

The optimum germination temperature of afterripened redbud seed was 70° F. Some germination occurred at a temperature as low as 33° F. Germination at 100° F. was slow and the seedlings produced and grown at that temperature either became unhealthy and abnormal in appearance or rotted completely.

Vitamin B_1 had no appreciable effect either on afterripening or germination

Ten days of dry storage of fully afterripened seed at 75° to 85° F. reduced the ability of the seed to germinate from the original of 96 percent to 90 percent.

Catalase activity increased during the progress of afterripening.

Catalase activity decreased steadily during the late stages of ripening of the seed.

LITERATURE CITED

(1) AFANASIEV, M.
1937. A PHYSIOLOGICAL STUDY OF DORMANCY IN SEED OF MAGNOLIA ACUMINATA. N. Y. (Cornell) Agr. Expt. Sta. Mem. 208, 37 pp., illus.

(2) ———
1939. GERMINATION OF REDBUD SEED. Amer. Nurseryman 69: [3].

(3) ——— and CRESS, M.
1942. PRODUCING SEEDLINGS OF EASTERN RED CEDAR (JUNIPERUS VIRGINIANA L.) Okla. Agr. Expt. Sta. Bul. B–256, 21 pp., illus.

(4) DAVIS, W. E.
1926. THE USE OF CATALASE AS A MEANS OF DETERMINING THE VIABILITY OF SEEDS. Boyce Thompson Inst. Prof. Paper 2, 12 pp. illus.

(5) ECKERSON, S.
1913. A PHYSIOLOGICAL AND CHEMICAL STUDY OF AFTER-RIPENING. Bot. Gaz. 55: 286–299.

(6) ENGSTROM, H. E., and STOECKELER, J. H.
1941. NURSERY PRACTICE FOR TREES AND SHRUBS SUITABLE FOR PLANTING ON THE PRAIRIE-PLAINS. U. S. Dept. Agr. Misc. Pub. 434, 159 pp., illus.

(7) HAUT, I. C.
1938. PHYSIOLOGICAL STUDIES ON AFTER-RIPENING AND GERMINATION OF FRUIT-TREE SEEDS. Md. Agr. Expt. Sta. Bul. 420, 52 pp., illus.

(8) HURST, W. M., HUMPHRIES, W. R., and MCKEE, R.
1935. BARREL AND DISC SEED SCARIFIERS. U. S. Dept. Agr. Dept. Cir. 345, 23 pp., illus.

(9) MCKEEVER, D. G.
1937. A NEW BLACK LOCUST SEED TREATMENT. Jour. Forestry 35: 500–501.

(10) PACK, D. A.
1921. AFTER-RIPENING AND GERMINATION OF JUNIPERS SEEDS. Bot. Gaz. 71: 32–60, illus.

(11) RHINE, L. E.
1924. DIVERGENCE OF CATALASE AND RESPIRATION IN GERMINATION. Bot. Gaz. 78: 46–67, illus.

(12) STOECKELER, J. H., and BASKIN, L. C.
1937. THE DENBIGH DISC SCARIFIER, A NEW METHOD OF SEED TREATMENT. Jour. Forestry 25: 396–398, illus.

(13) SWINGLE, C. F.
1939. SEED PROPAGATION OF TREES, SHRUBS, AND FORBS FOR CONSERVATION PLANTING. U. S. Dept. Agr. Soil Conserv. Serv. TP–27. 198 pp. [Mimeographed.]

(14) TOUMEY, J. W., and KORSTIAN, C. F.
1931. SEEDING AND PLANTING IN THE PRACTICE OF FORESTRY. Ed. 2, rev. and enl., 507 pp., illus. New York and London.

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., DECEMBER 1, 1944 No. 11

DEVELOPMENT AND SURVIVAL UNDER OUTDOOR CONDITIONS OF EGGS AND LARVAE OF THE COMMON RUMINANT STOMACH WORM, HAEMONCHUS CONTORTUS[1]

By A. G. DINABURG

Assistant zoologist, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Pasture rotation has long been one of the measures recommended for the control of roundworm parasites of sheep and other ruminants. This method of control must be based on a knowledge of the length of time that pasture previously occupied by parasitized ruminants should be rested before susceptible animals can be grazed on it without danger of acquiring nematode parasites. To obtain this knowledge, the period of survival of the free-living stages of these nematodes must be known.

Studies that have been made on the free-living stages of the common stomach worm, *Haemonchus contortus*, an important and widespread parasite of sheep and other ruminants, are of two major groups, namely, (1) those concerned with the effects of various environmental factors on the eggs and larvae under laboratory and field conditions and (2) those having to do with the survival of infective larvae on pastures.

REVIEW OF LITERATURE

Ransom (*7*)[2] found that "the eggs and newly hatched embryos possess little powers of resistance, and many die if subjected to freezing or drying," whereas infective larvae exposed outdoors in feces during the winter survived repeated freezing and thawing for 12 weeks and were also resistant to drying for 35 days.

Veglia (*9*) reported the results of both laboratory and field experiments on the survival of the eggs and larvae of *Haemonchus contortus*. This author found that under laboratory conditions temperatures between 68° and 95° F. favored the development of eggs in cultures, whereas at 46° only 30 percent of the eggs had reached the second larval stage after 21 days, and at 32° all the eggs were killed after 48 hours. Exposure of the eggs to 32° or 39° for 24 hours, followed by a return to 75°, killed many of them. Temperatures above 95° became increasingly unfavorable to the development of the eggs, but infective larvae resisted temperatures of 122° for about 2 hours, 108° for a maximum of a month, and 32° for 6 to 7 months. Drying

---

[1] Received for publication March 16, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 433.

prevented the development of eggs and larvae and killed infective larvae within a few days under laboratory conditions. However, eggs containing embryos resisted drying in air better than did eggs in the early stages of development. Although moisture was necessary for the development of eggs and larvae in cultures, an excess of moisture inhibited their development. Infective larvae, however, survived well in water.

The effects of sunlight and moisture on the survival of eggs and larvae outdoors were also studied by Veglia. Some of the experiments were conducted under artificial conditions; for example, cultures of feces were kept under a glass jar or were watered at regular intervals to reduce drying. From the results of these experiments the following inferences were made. Constantly warm and moist or cloudy weather was favorable to the development of a majority of the eggs, but constantly warm, dry weather, such as occurred during periods of drought, was unfavorable. Constantly dry, cold weather, expecially if the temperature fell to freezing or below for long uninterrupted periods, killed both eggs and larvae. No definite information was available on the effect of variable weather. A high percentage of eggs and freshly hatched larvae were killed if the first 2 days after their deposition on soil were dry and warm, or even when the first day was cloudy, provided the soil was also dry. On the other hand, if the feces were dropped after a heavy rain, numerous eggs survived, especially in grass, even if the first day was sunny and warm. The nature of the pasture and that of the soil itself were factors to be considered. Most of the eggs passed by infected animals failed to reach maturity.

The results of Veglia's experiments were frequently described in general terms, such as "few larvae survived." In other instances the percentages of eggs or larvae surviving or dead were given without the initial or final numbers involved, so that it is difficult to determine whether a sufficiently large number of eggs or larvae was used on which to base conclusions.

Guberlet (*4*) found larvae of *H. contortus* alive after 4 to 5 months in soil and feces cultures. He also found 30 larvae in a pool of water in an infected pasture but gave no further details.

Mönnig (*6*) found that the preinfective stages of *H. contortus* were not resistant to drying, but infective larvae when air-dried indoors survived for a maximum of 4½ months and when exposed to the sun in a mixture of mud and water they survived about 2½ months. Outdoors, the infective larvae survived for as long as 9½ months on grass and clay soil artificially kept moist from below and sheltered from direct sunlight, but survived less than 11 weeks when exposed on black clay soil and grass to dry summer conditions in the Union of South Africa. Data on temperature, humidity, and number of larvae initially used or recovered were not given.

Bozevich (*1*) noted that some larvae exposed on soil to natural weather conditions were alive after 7 months. Eggs refrigerated indoors at −2.2° C. (28° F.) in soil cultures were killed in 6 to 8 hours, depending on the soil type. First-stage larvae in similar cultures were dead after 28 hours, whereas second-stage larvae were dead after a week at 0° C. (32° F.).

Fallis (*3*) found that all eggs of *H. contortus* died after exposure to −10° C. (14° F.) for 3 to 6 days, whereas the controls, incubated at room temperatures, hatched.

Taylor (*8*) made observations on the longevity of various strongyloid larvae from sheep, rabbits, and horses, the larvae having been placed on grass grown in boxes and kept in an open field. He found that the majority of the larvae recovered from the grass died within the first few weeks of exposure and that only a small proportion remained alive for more than 63 days. Since his experiment with sheep parasites dealt with an unspecified mixture of trichostrongylid larvae, it is not possible to utilize his results in attempts to control any particular species.

Kauzal (*5*), in a brief progress report on work done in Australia, stated that very short periods of desiccation in sunlight were fatal to eggs of both *H. contortus* and *Trichostrongylus* spp., and that some days of continuous rainy or cloudy weather were necessary for the development of a high proportion of larvae of either species. He also reported that the survival period of infective larvae, when exposed to desiccation, is less than previously supposed, and that a rest of 1 to 2 months during periods of continuously dry weather may serve to free a pasture completely of larvae of *H. contortus*.

From the foregoing survey of the literature it is apparent that previous work on the development and survival of eggs and larvae of *H. cortortus* under laboratory or field conditions has been concerned mainly with the discovery of the maximum period of survival under various conditions and, with the exception of Veglia's work, these studies have been primarily qualitative. A quantitative study of the rates of development and survival of the various free-living stages under the influence of natural weather conditions is described in the present paper. This work was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

MATERIALS AND METHODS

Experimental infections with *Haemonchus contortus* were maintained continuously in young rams that had been raised free of nematodes, except for occasional infections with *Strongyloides papillosus*. These rams were kept indoors in concrete-floored pens that were cleaned daily. Their feces were collected in cloth bags, held firmly against the hindquarters of the sheep by a cloth harness. Each bag was emptied daily into a pail used exclusively for a particular sheep.

For use in an experiment, the pellets passed during the previous 24-hour period were finely ground in a mortar. The entire mass was then thoroughly stirred, and sixteen 50-gm. samples were weighed out. Twelve samples were exposed outdoors as described below and the remaining four samples were used as controls. The control samples were put into separate, covered pint jars and kept at room temperature in a dark closet. A modified Stoll dilution egg count was also made.

The experiments, totaling 30, were performed from August 16, 1939, to October 29, 1940. The first six experiments were begun from 5 days to 2 weeks apart, but after the sixth, they were begun 2 weeks apart except for a gap between experiments 28 and 29. Each experiment was made as follows:

A sandy loam was treated with steam in a closed box to kill any free-living or parasitic nematodes that might be present. Samples of this heated soil showed no nematodes when examined. Twelve unglazed flowerpots, about 8½ inches high and 8 inches in diameter at the top, were filled with the soil to within 1½ inches of the top and were then planted with seeds of Italian ryegrass (*Lolium multiflorum*) at the rate of about 1.7 gm. to each pot. This grass was raised either in the laboratory or outdoors, depending on the season, to a height of about 3 to 4 inches. The pots were then buried in the ground so that the soil inside and that outside the pots were at about the same level, leaving a rim of about 1½ inches to prevent the feces, eggs, and larvae from washing away. The pots were placed in groups of four in three different locations—full sunlight, partial sun and shade, and full shade. In order to maintain these exposures, the pots were moved to various locations during the year as the sunlight and shade shifted. Those pots exposed to partial sun and shade received sunlight until about noon and shade in the afternoon. After a week in these positions, to permit the soil moisture and temperature to approach those of the surrounding soil, the pots were infected with the twelve 50-gm. samples of feces, which were evenly distributed over the surface of the pots at the base of the grass.

After a given period of exposure outdoors, a pot from each of the three locations was taken into the laboratory and examined to obtain information on the survival of the eggs and larvae. The grass was clipped as close to the soil as possible, placed in a beaker, and left covered with water for 24 hours, after which the washings were examined for the presence of larvae. The feces and the surface layer of soil were scraped up, weighed, and a maximum of 100 gm. was put into the Baermann apparatus. To obtain better sampling of the soil in the pot successive 1-inch layers of soil were removed, weighed, stirred, and a 100-gm. sample from each layer was put into the Baermann apparatus. At least the top 3 inches of soil and in most cases the entire contents of the pot were thus sampled.

The specifications and use of the Baermann apparatus employed in the present experiments have already been given by the writer (*2*). After 48 hours, samples of about 50 to 60 cc. were drawn from the apparatus and examined. When few larvae were present in the sample, all were counted; otherwise they were counted by a combination of subsampling and dilution methods. Since previous experiments indicated that few dead larvae would pass through the Baermann apparatus and be found in the 50- to 60-cc. sample, the dead larvae found in the Baermann samples were considered to have died after going through the apparatus and were added to the number of live larvae found at the time of examination. In most experiments, the live larvae outnumbered the dead ones. For comparison, only the live larvae in the control cultures were counted.

One of the four cultures raised as controls in the laboratory was examined at each interval by a combination of dilution and subsampling methods. The feces and the larvae on the walls and cover of the culture jar were washed into a calibrated beaker and diluted to a known volume of feces and water. After this mixture had stood for several minutes to permit the dried larvae to revive, it was stirred vigorously and a subsample was taken from it. At least three, and in most cases four, subsamples were taken from each suspension. From the number of larvae in these subsamples the total in 50 gm. of feces was calculated, and the average of the four controls was then used for comparison with the total number recovered from the pots after exposure outdoors.

The presence of viable but still unhatched eggs after a given period of exposure was determined by salt flotations on samples of feces taken from each pot at the time of collection. Moving tadpole or embryo stages within the eggs were the test signs of viability. In addition to these examinations, the hatchability of eggs after exposure late in the fall, in the winter, and early in the spring was determined. In the ninth to twenty-first experiments, 5-gm. samples of feces were taken from each pot after exposure and cultured in the laboratory in Syracuse watch glasses resting in a covered water trap. After an interval of at least a week, the contents of the watch glass and water trap were examined for live larvae.

Air temperatures were measured with a U-type combination maximum-minimum thermometer hung 4 feet above the ground on the north side of a tree trunk. It was read each morning, except on Sundays and holidays, and reset. Measurements of the temperature immediately under the surface of the soil exposed to full sunlight or full shade near the pots were begun in June 1940. Rainfall data were obtained from a station located at the Beltsville Research Center about 1.3 miles from where the experiments were conducted. The gage at this station was read about 9 o'clock each morning.

SURVIVAL OF THE EGGS

VIABILITY OF EGGS AS DETERMINED BY SALT FLOTATIONS

Of the samples from 360 pots examined by the salt flotation method, 44, or 12 percent, in 13 experiments contained viable eggs. These positive experiments were subdivided into 2 groups according to whether they were exposed, during the first period, to mean maximum temperatures above or below 70° F. The low-temperature group included experiments 9, 10, 12, 17, 19, 20, 21, and 30; the high-temperature group, experiments 3, 4, 6, 25, and 28. Further details of these experiments are given in table 3. The results of the salt-flotation examinations are given in table 1, which indicates that unhatched eggs do not remain viable outdoors for more than 13 to 20 days. Within this interval of 20 days, maximum temperatures below 70° favor the longer persistence of viability of eggs than temperatures above 70°.

TABLE 1.—*Viability of eggs of Haemonchus contortus after outdoor exposure as shown by their recovery in salt flotations*

Period of exposure (days)	Total pots examined	Pots containing viable eggs after exposure to mean maximum temperature—		Viable eggs based on total number examined
		Below 70° F.	Above 70° F.	
	Number	*Number*	*Number*	*Percent*
3	66	14	12	39.8
6	90	14	2	17.2
13	90	2	0	2.3
20	90	0	0	0
27	24	0	0	0

HATCHING OF EGGS IN LABORATORY CULTURES AFTER OUTDOOR EXPOSURE

Of a total of 150 laboratory cultures in 13 experiments (Nos. 9 to 21), after outdoor exposure 18 cultures from 6 experiments contained some live larvae. In these 6 experiments the mean maximum air temperatures ranged from 39° to 70° F. during the first 13 days of outdoor exposure. The results of the 18 positive cultures are given in table 2; further details of the experiments, in table 3.

In table 2, the total number of eggs in each pot that could hatch into larvae was computed from the number of larvae hatching in the culture from that pot and added to those for the other two pots to obtain the total number of eggs surviving a given outdoor exposure in an experiment. For comparison with the controls, this total was divided by three times the number of larvae found in parallel control cultures as given in table 3.

The data in table 2 show that eggs do not hatch after exposure to mean maximum temperatures below 70° for more than 6 days. Furthermore, of the 13 experiments that were made, the proportion of eggs that hatched after outdoor exposures of 3 or 6 days, by comparison with the number that hatched in the controls, was very low in 6 experiments and negative in 7.

TABLE 2.—*Effect of previous outdoor exposure on hatching of Haemonchus contortus eggs in 6 experiments*

Experiment No.	Larvae in control cultures	Eggs hatching in laboratory cultures, compared with those in controls, after exposure of—				
		3 days	6 days	13 days	20 days	27 days
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
9	242,250	1.7	0	-----	-----	-----
10	66,300	.8	0	0	0	-----
12	308,400	-----	.07	0	0	0
17	324,750	-----	.005	0	0	0
20	159,600	1.1	.3	0	0	-----
21	105,750	.3	0	0	0	-----

DEVELOPMENT OF LARVAE

The effect of outdoor exposure in various locations on the development of the eggs, as determined by the percentages of larvae recovered from the pots, is shown in table 3. The larvae recovered after 3 or 6 days' exposure were mostly preinfective, whereas those exposed for longer periods were mostly infective.

The total rainfall for the several periods of exposure was calculated by somewhat different methods. Since Veglia (*9*) concluded that the soil moisture as well as the weather during the early days of exposure affected the survival and development of the eggs, some method of taking this first factor into account was necessary. As no means of measuring soil moisture directly was available, the amount of rainfall on the pots during the 7-day outdoor conditioning period was taken as a rough but practical indicator of the soil moisture at the time of exposure of the eggs. This 7-day total was added to that for the first 3-day period of exposure to obtain the rainfall data pertinent to the 3-day period. The total rainfall during the subsequent exposure period and in the initial 6-day exposures of winter experiments 11 to 18 was found from the amount that fell from the end of the previous period to the end of the period in question.

TABLE 4.—*Summary of table 3, showing effect of temperature and rainfall during the early exposure period on the development of larvae*

Location of pots	Period of exposure	Larvae recovered after exposure of experimental pots to mean maximum temperature and rainfall of —				
		26° to 65° F.[1]	66° to 84° F.			86° to 93° F.
		0 to 3.02 inches	0 inch	0.12 to 0.72 inch	0.88 to 2.99 inches	0.26 to 1.57 inches
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Shade	3	0.001	0.003	14.0	32.0	3.0
	6	0	.0002	1.0	23.0	9.0
	13	0	.002	.3	18.0	8.0
	20	0	.002	.09	11.0	4.0
Partial shade	3	.003	.001	14.0	29.0	.1
	6	0	0	1.0	20.0	.1
	13	0	.02	.5	16 0	.006
	20	0	.01	.3	21.0	.04
Sun	3	.001	.001	3.0	12.0	0
	6	.0002	.01	.3	6.0	.002
	13	0	.02	.08	4.0	.03
	20	0	.005	.003	6.0	0
Number of experiments in group		13	4	5	4	4
Total number of larvae in controls for group		508,500	661,500	1,317,500	885,700	64,700

[1] The pots in experiments 11 to 18, inclusive, were first examined after a 6-day exposure.

Of 13 experiments in which pots were exposed to mean maximum temperatures, during the first exposure period, of less than 65°, only 3 yielded a total of 30 preinfective larvae after 3 days and 1 preinfective larva after 6 days. No larvae were recovered after longer exposures. The amount of rainfall varied widely, with no apparent effect on development of the larvae.

The 13 experiments in which the pots were exposed to initial maximum temperatures of 66° to 84° fall into 3 rainfall subdivisions. In the first subdivision, with no rainfall, are 4 experiments in which the numbers and proportions of larvae recovered after all exposures were very low.

In the second rainfall subdivision, containing 5 experiments, the percentages recovered were generally higher than those in the first. Although these percentages were variable, there was no consistent increase in recoveries with increasing rainfall in individual experiments. On the other hand, the degree of exposure to sunlight seemed to affect the recoveries, for the pots exposed to full sunlight yielded lower percentages than those exposed either to full shade or partial sun and shade. After exposure for 6 days there was a sharp drop in the percentages recovered, except in experiment 30, which had a higher recovery at 6 days than at 3 days. The recoveries of infective larvae after 13- and 20-day exposures were below 1 percent of the controls, except for 1 pot.

In the third and highest rainfall subdivision are four experiments which as a group had the highest percentage of both preinfective and infective larvae developing outdoors. Lower percentages were recovered from pots exposed in the sun than from the other two locations, from which recoveries were generally in rather close agreement. The comparatively high average for the 20-day period of exposure in partial shade was due to the results obtained in experiment 3. The recoveries from experiment 25, much lower than those from the others, cannot be explained on the basis of either temperature or rainfall.

In the high-temperature group, containing four experiments, the recoveries after exposure in partial shade were much lower than those in the middle range of temperatures with some accompanying rainfall, and there was an almost complete absence of larvae in the pots exposed to the sun. An estimate of the temperatures just under the surface of bare soil exposed to the sun during various seasons is provided by the measurements, begun with experiment 25, shown in table 5. The corresponding temperatures taken immediately under the bare soil in the shade approximated closely those in the air in the shade.

TABLE 5.—*Means of maximum temperatures taken immediately under the surface of bare soil in full sunlight and in the air in the shade*

Period	Temperatures taken in the soil exposed to the sun	Temperatures taken in the air in the shade	Difference	Period	Temperatures taken in the soil exposed to the sun	Temperatures taken in the air in the shade	Difference
1940				*1941*			
	° F.	*° F.*	*° F.*		*° F.*	*° F.*	*° F.*
June[1]	121.6	83.6	38.0	January	43.6	39.0	4.6
July	126.7	87.8	38.9	February	50.5	40.0	10.5
August	110.6	81.6	29.0	March	65.5	49.9	15.6
September	97.9	75.8	22.1	April	105.5	73.1	32.4
October	78.3	63.0	15.3	May	126.4	80.8	45.6
November	61.0	52.9	8.1	June	112.9	78.5	34.4
December	53.6	47.6	6.0				

[1] Temperatures for June 26 to 30 only.

As previously stated, Veglia (*9*) found that temperatures above 95° F. become increasingly unfavorable to the development of eggs and that infective larvae resist temperatures of 122° for only 2 hours. Although the pots had a cover of ryegrass, the high temperatures undoubtedly were at least partly responsible for the faster scorching of the grass and the faster drying of the soil and feces observed in the pots exposed in the sun during the hot summer periods and for the resultant low recoveries of larvae from these pots.

DISCUSSION

Before applying the results of the above experiments, it is necessary to determine how closely the field conditons are indicated by the present measurements of weather factors and of their effect on the preparasitic stages of *Haemonchus contortus*.

The maximum air temperatures taken in the shade, used throughout the present experiments, approximate closely those taken immediately below the surface of the soil in the shade but are lower than the soil temperatures in the sun. In addition to this varying exposure to the sun at different seasons, the difference between air temperatures and those on or near the surface of the soil may be affected by the situation and slope of the land, the soil type, and the amount and density of plant cover. In view of these considerations, maximum air temperatures in the shade indicate best the soil-surface temperatures in continuous shade but underestimate those in the sun, where higher temperatures might favor more rapid development of the eggs in cool weather or destruction in warm weather.

Since the moisture content of the environment of the eggs and larvae could not be measured directly, the amount of rainfall was taken as a measure of the water available for keeping the eggs and larvae moist in their environment. It is recognized that many variable processes and factors may intervene between a given rain and its appearance as moisture in the environment, but for the present, rainfall is taken as an over-all, rough, but practical measure of this factor. As in the case of temperature, local differences in moisture content among pots in different situations may be created by differences in exposure to sun or shade. In addition, differences in rainfall between the site of the experiments and the site of the rain gage, while suspected, could not be measured. However, with all these shortcomings, the present methods of measurement of weather factors have the merit of being easily made under field conditions.

The effect of these weather factors on the eggs and larvae was measured mainly by comparing the number of larvae recovered after outdoor exposure with those developing in the controls. Since the larvae exposed outdoors were recovered in part by means of the Baermann apparatus and then counted, whereas those in the controls were counted directly in water suspension, the comparability of the results of these two methods must be checked.

A study of the effectiveness of the Baermann apparatus in the recovery of infective larvae of *H. contortus* by the writer (*2*) shows that both the number of larvae put into the apparatus and the type of substrate through which they must pass, affect the efficiency of recovery. For example, with a substrate of 100 gm. of the same sandy loam and the same Baermann apparatus used in the present experiments, it was found that an average of 11 percent was recovered when 150 to 420 larvae were put into the apparatus, and 33 percent when 600 to 95,400 larvae were used. With a substrate of 100 gm. of ground sheep feces, these recoveries were 5 and 11 percent, respectively. In addition, the apparatus was shown to perform inconsistently from one experiment to another.

Because of these losses, the number of larvae recovered with the apparatus in the above experiments is not directly comparable with the number in the controls. Correction factors based on the efficiency experiments cannot be applied to the present recoveries with the apparatus because these factors are based on recoveries through homogeneous substrates of soil or feces, whereas in the present experiments 88 percent of all larvae recovered came from the surface layer, which was a variable mixture of soil and feces. However, it is safe to say that the total number of larvae surviving outdoor exposure is higher than the number recovered, especially when the recoveries are low. Consequently the percentages of controls recovered, with which the present paper deals, give a minimum estimate of the proportions of larvae surviving outdoor exposure.

The data on the effect of the early weather conditions on the development and survival of the eggs and larvae of *H. contortus* show that temperature and rainfall exchange places as limiting factors in the development of the eggs at maximum temperatures of about 65° F. When the mean maximum temperature in the shade of the first exposure period is below 65°, few if any eggs hatch no matter how much rain falls, and no infective larvae are recovered after 20 days outdoors. With mean maximum temperatures between 66° and 84°, the development of the eggs into larvae and the survival of the larvae increase with increasing amounts of rainfall during the 7 days before and 3 days after exposure of the eggs. With temperatures above 86°, a range of 0.26 to 1.57 inches of rainfall is accompanied by a moderate degree of survival only in exposures to shade.

The amount of exposure to the sun, when the mean maximum temperatures range above 65°, also affects the degree of development and survival. The lowest recoveries were obtained from the pots exposed in full sunlight. About the same recoveries were obtained from the pots exposed in partial shade as from those exposed in full shade in the middle temperature range. However, in the high temperature range, the recoveries from the pots in partial shade were consistently far lower than those from the pots in full shade, possibly as a result of higher soil temperatures.

Of these two limiting factors, temperature is more reliable than rainfall for the designation of definite periods of the year as unfavorable for the development of the eggs of *H. contortus*, since seasonal trends are more stable and predictable for temperature than for rainfall conditions, in a temperate climate. Further, if the maximum air temperatures are constantly below 65° F., no development of eggs will occur regardless of the amount of rainfall. On the other hand, above 65° only consistently dry periods can be used to designate intervals unfavorable for development.

SUMMARY AND CONCLUSIONS

Development of the eggs and survival of the infective larvae of *Haemonchus contortus* under the influence of natural weather conditions were studied by exposing feces containing eggs of this nematode outdoors in flowerpots under a cover of ryegrass. Twelve pots, examined in groups of 3 at 4 intervals up to 27 days, comprised 1 experiment. The experiments, totaling 30, were conducted from August 1939 to October 1940 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. The following conclusions are based mainly on a comparison of the number of larvae recovered after exposure outdoors with the number developed in laboratory controls and on the relationship of this ratio with the accompanying outdoor air-shade temperature and rainfall data.

When eggs of *H. contortus* were exposed outdoors for 3 days or more to mean maximum temperatures below 65° F., few preinfective larvae—those recovered after 3 or 6 days' exposure—and no infective larvae—those recovered after 13 or more days—were found during the experiments, regardless of the amount of rainfall.

With mean maximum temperatures between 66° and 84° during the first 3 days, the amount of rainfall in the week preceding and the 3 days following exposure of the eggs considerably affected the degree of their development. After 13 to 20 days outdoors, with no rainfall, infective larvae recovered, based on the number of controls, at no time exceeded an average of 0.01 percent; with 0.12 to 0.72 inch and 0.88 to 2.09 inches of rainfall, the highest averages were 0.5 and

21 percent, respectively. Higher percentages of preinfective larvae were recovered.

With mean maximum temperatures between 86° and 93° and 0.26 to 1.57 inches of rainfall, the highest recovery of infective larvae was 8 percent, after 13 days.

Exposure to full sunlight resulted in lower yields of larvae than exposure to full shade or partial shade. In the middle temperature range, recoveries from exposure to full and partial shade were about the same. In the upper temperature range recoveries from the former were consistently higher than from the latter.

Recoveries from the pots provide only a minimum estimate of the number of larvae surviving outdoor exposure because of the losses in the Baermann apparatus.

Eggs do not survive exposure to mean maximum temperatures below 70° for more than 6 to 13 days, as shown by cultures, or for more than 13 to 20 days, as shown by salt flotations. Above 70°, salt flotations indicate that eggs do not survive more than 6 to 13 days outdoors.

In designating large periods of the year as unfavorable for the survival and development of the eggs of *H. contortus*, temperature is a more reliable weather factor than rainfall.

LITERATURE CITED

(1) Bozevich, J.
1930. PRELIMINARY REPORT ON THE VIABILITY OF HAEMONCHUS CONTORTUS LARVAE ON SOIL EXPOSED TO WEATHER. Helminthol. Soc. Wash. Proc. 128; Jour. Parasitol. 17: 53-54.

(2) Dinaburg, A. G.
1942. THE EFFICIENCY OF THE BAERMANN APPARATUS IN THE RECOVERY OF LARVAE OF HAEMONCHUS CONTORTUS. Jour. Parasitol. 28: 433-440, illus.

(3) Fallis, A. M.
1938. A STUDY OF THE HELMINTH PARASITES OF LAMBS IN ONTARIO. Roy. Canad. Inst. Trans. 22 (1): 81-128, illus.

(4) Guberlet, J. E.
1921. STOMACH WORMS IN SHEEP. Okla. Agr. Expt. Sta. Bul. 137, 16 pp., illus.

(5) Kauzal, G.
1936. OBSERVATIONS ON VIABILITY AND RESISTANCE TO DESICCATION OF EGGS AND LARVAE OF HAEMONCHUS CONTORTUS AND TRICHOSTRONGYLUS SPP. Austral. Council Sci. and Indus. Res., Ann. Rpt. 10: 33.

(6) Mönnig, H. O.
1930. STUDIES ON THE BIONOMICS OF THE FREE LIVING STAGES OF TRICHOSTRONGYLUS SPP. AND OTHER PARASITIC NEMATODES. Union So. Africa Dept. Agr. Rpt. Dir. Vet. Serv. and Anim. Indus. 16:175-198.

(7) Ransom, B. H.
1906. THE LIFE HISTORY OF THE TWISTED WIREWORM (HAEMONCHUS CONTORTUS) OF SHEEP AND OTHER RUMINANTS. U. S. Dept. Agr., Bur. Anim. Indus. Cir. 93, 7 pp., illus.

(8) Taylor, E. L.
1938. OBSERVATIONS ON THE BIONOMICS OF STRONGYLOID LARVAE IN PASTURES: I.—THE DURATION OF THE INFECTION IN PASTURE HERBAGE. Vet. Rec. 50: 1265-1272, illus.

(9) Veglia, F.
1916. THE ANATOMY AND LIFE HISTORY OF THE HAEMONCHUS CONTORTUS (RUD.) Union So. Africa Dept. Agr. Rpt. Dir. Vet. Res. 3 and 4: 347-500.

MEIOTIC STUDIES OF CROSSES BETWEEN FRAGARIA OVALIS AND × F. ANANASSA[1]

By LeRoy Powers

Formerly *senior geneticist,*[2] *Division of Fruit and Vegetable Crops and Diseases Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultura Research Administration, United States Department of Agriculture*

INTRODUCTION

The value of the native Rocky Mountain strawberry (*Fragaria ovalis* (Lehm.) Rydb.) for hybridizing with cultivated varieties (× *F. ananassa* Duch.) to develop new varieties having greater winter hardiness has long been recognized (*2, 8, 10*).[3] However, the karyological investigations of Longley (*17*), East (*4, 5*), Mangelsdorf and East (*18*), Kihara (*13*), Yarnell (*29, 30*), Ichijima (*11*), Fedorova (*6*), Lilienfeld (*15, 16*), Kuz'min (*14*), Rozanova (*25*), and Dogadkina (*3*) show the importance of meiotic studies as an aid to any breeding program involving wide crosses between polyploid species and forms within the genus *Fragaria.* The researches of these investigators emphasize particularly the part that asynapsis, conjugation of more than two chromosomes to form multivalents during meiosis, and type of chromosome conjugation (autosyndesis, allosyndesis, or a combination of the two) may play in the breeding of improved varieties.

The importance of asynapsis and of the conjugation of more than two chromosomes to form multivalents during meiosis lies in the effect that they have upon fruitfulness. By adversely affecting fruitfulness asynapsis of the chromosomes during meiosis, if of frequent occurrence, may be one of the major factors contributing to the failure of a breeding program. Likewise, if homology exists between different genoms coming from the same polyploid species, as well as between genoms coming from different polyploid species, conjugation of more than two chromosomes to form multivalents might be of frequent occurrence. If such were the case, one would expect fruitfulness to be reduced materially, possibly to the extent that the accomplishment of the objectives of the breeding program would be threatened.

Turning to the type of conjugation of the chromosomes, if autosyndesis (the pairing, in a polyploid, of chromosomes derived from the same parent) was occurring, then variation in the F_2 population would be limited largely to that taking place within the F_1 hybrid, and the chances for recombining any of the desirable characters of both parents into a single segregate would be decidedly reduced. On the other hand, allosyndesis (pairing in a polyploid of chromosomes derived from opposite parents) would allow for the maximum segregation of the genes differentiating the interspecific characters. A combination of autosyndesis and allosyndesis would result in an

---

[1] Received for publication May 11, 1943.

[2] Now principal geneticist, Special Guayule Research Project, U. S. Department of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 447.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 11
December 1, 1944
Key No. G-1318

intermediate condition as regards the possibilities for maximum segregation of these same genes. Consequently, information concerning the type of conjugation of the chromosomes during meiosis of the F_1 hybrids would be of considerable value in planning and conducting a breeding program.

The objectives of the studies herein reported were (1) to determine whether asynapsis and the association of more than two chromosomes to form multivalents during meiosis occur frequently enough to affect a breeding program adversely and (2) to ascertain the type of conjugation that occurs during meiosis of the F_1 plants.

REVIEW OF LITERATURE

With the exception of a few varieties produced by Georgeson (*8*), the cultivated strawberries have descended from crosses involving *Fragaria virginiana* and *F. chiloensis*, both of which are octoploids. The basic number of chromosomes in *Fragaria* is seven; hence, the chromosomes of the cultivated strawberry are comprised of four genoms each having seven pairs of chromosomes. The best available information as to the relation between these four sets of chromosomes and between the chromosomes of different species is that obtained from the genetic and karyological investigations of crosses between species of different chromosome numbers and, to a more limited extent, between species of the same chromosome number.

The investigations of Longley (*17*), East (*4*, *5*), Mangelsdorf and East (*18*), Kihara (*13*), and Yarnell (*29, 30*) showed that at least some chromosomes of the four genoms of seven pairs of chromosomes are homologous. Yarnell (*29, 30*), from cytological and genetic studies, came to the conclusion that the four sets of chromosomes of the octoploid had a common origin and that corresponding chromosomes of each set were originally homologous. After examining microsporocytes from diploid-tetraploid and diploid-octoploid crosses, Yarnell reported not only pairing between chromosomes from both parents but also autosyndesis among the remaining genoms of the tetraploid parent and the remaining sets of the octoploid parent. He found that in many cases the counts at first metaphase indicated complete pairing, involving the nonhomologous chromosomes of the extra genom, and, in addition, he found secondary association taking place between disomes.

The findings of Ichijima (*11*), Fedorova (*6*), Lilienfeld (*15, 16*), Kuz'min (*14*), Rozanova (*25*), and Dogadkina (*3*) in regard to the homologous relations between the chromosomes of the octoploid forms are well summarized by the following statement from Rozanova (*25*):

> . . . it may be deduced that the evolution of species of *Fragaria* has proceeded in the direction of autopolyploidy or close allopolyploidy. From this it follows that the hypothesis as to the origin of cultivated varieties from a cross between *F. virginiana* and *F. chiloensis* needs supplementing to the extent of stating that *F. virginiana* and *F. chiloensis* are also probably autopolyploids or close allopolyploids with homologous genoms.

From the above investigations it is evident that the haploid chromosome complement of the octoploid forms of *Fragaria* may be symbolized as follows:

F. ovalis—Ao_1, Ao_2, Ao_3, Ao_4
F. chiloensis—Ach_1, Ach_2, Ach_3, Ach_4

F. virginiana—Av_1, Av_2, Av_3, Av_4
× *F. ananassa*—Acv_1, Acv_2, Acv_3, Acv_4

These formulas are closely patterned after those given by Rozanova (*25*). The *A* is used to show that all the chromosomes of one species are homologous with the chromosomes of any other and that a certain amount of homology exists between genoms within a species. *Acv* indicates that the genoms of × *F. ananassa* through hybridization descended from those of *F. chiloensis* and *F. virginiana.*

From the studies of the various workers cited, it can be seen that there are a number of possibilities as regards chromosome behavior during meiosis in the F_1 hybrids between *Fragaria ovalis* and × *F. ananassa.* Asynapsis may or may not be of frequent occurrence. Associations of more than two chromosomes to form multivalents may be the rule, or such associations may occur occasionally or only very infrequently. Finally, the four genoms from *F. ovalis* may pair during meiosis with the four genoms from the cultivated varieties (allosyndesis); two genoms from *F. ovalis* may pair with two other genoms from that species and hence two genoms of × *F. ananassa* with two other genoms of that species (autosyndesis); and both allosyndesis and autosyndesis may occur either at random or in disproportionate frequencies.

EXPERIMENTAL MATERIAL AND DESIGN

The parents and F_1 hybrids were used in the studies. The design of the experiment was that of a randomized complete block and the variates were as follows:

Group I (collections of *Fragaria ovalis*): A, 37501; B, 361477; C, 36979.
Group II (F_1 hybrids): A, Dorsett × 37501; B, Gem × 361477; C, Fairfax × 36979.
Group III (varieties of × *F. ananassa*): A, Dorsett; B, Gem; C, Fairfax.

Each group hereafter will be called a species containing three variates. Although group II cannot strictly be classed as a species, it is clear that any differences between this group and either of the other groups must be due to genetic differences between groups I and III. Hence, from genetic considerations one is justified in considering the genetic variation between these three groups as due to inherent differences between species. It follows that, if the differences between the totals of these three groups can be attributed to chance deviations, it cannot be concluded, so far as these collections and varieties are concerned, that there are any differences between species.

The grouping on the basis of A, B, and C is as follows:

Group A: I, *Fragaria ovalis* (37501); II, F_1 (Dorsett × 37501); III, × *F. ananassa* (Dorsett).
Group B: I, *F. ovalis* (361477); II, F_1 (Gem × 361477); III, × *F. ananassa* (Gem).
Group C: I, *F. ovalis* (36979); II, F_1 (Fairfax × 36979); III, × *F. ananassa* (Fairfax).

Again, each group, which hereafter will be designated as a strain, contains three variates. The totals for groups A, B, and C will show statistically significant differences only if there are genotypic differences between the variates of group I, group III, or both I and III. Hence, one is justified in attributing any differences between A, B, and C to differences between strains.

The measure of asynapsis used in these studies was (1) the number of pollen mother cells in 100 observations showing some univalent chromosomes during metaphase I, (2) the number of cells in 100 observations showing at least 1 lagging chromosome during early telophase I, (3) the number of cells in 100 observations showing some chromosomes not on either equatorial plane during metaphase II, and (4) the number of cells in 100 observations showing some lagging chromosomes during early telophase II in any member of the potential tetrads. Thus, 4 different phases of meiosis were studied. In addition, the previously mentioned 100 pollen mother cells in metaphase I were examined to obtain some estimate of the frequency of occurrence of associations of more than 2 chromosomes during meiosis. Four plants of each variate were included in the studies, and 25 cells of each plant were examined. The 4 plants were from 4 different replicates.

With the present limited knowledge of the morphology of the strawberry chromosomes, it is not possible to determine through cytological examination of the stages of division whether autosyndesis, allosyndesis, or a combination of the two is occurring during meiosis. However, indirect but reliable evidence as to the type of conjugation that is occurring can be obtained by studying the means of certain characters of the parents and the hybrid populations.

The young anthers were killed in a solution of 3 parts of absolute alcohol to 1 part of glacial acetic acid and stored in 70-percent alcohol. Belling's (*1*) iron-acetocarmine method was employed in staining the material.

At this time it should be noted that the plants of any particular collection may not be alike genetically. For example, collection 37501 is composed of at least two strains, one of which has pistillate flowers while the other has perfect flowers. The strain possessing the perfect flowers was used in the cytological studies.

ANALYSIS OF THE DATA

Since the data were enumeration data, the method of analysis chosen was to partition χ^2 into its components (see Fisher, *7*) and calculate the heterogeneity χ^2 when it seemed desirable. However, the formulas developed and used in calculating the different χ^2's are not available elsewhere. Therefore, since they are of general application (particularly for enumeration data such as those obtained in germination studies), and since they materially simplify the calculations, detailed illustrations of their application are given.

The components of χ^2 together with their corresponding degrees of freedom are as follows:

Variation due to:	*Degrees of freedom*
Main effects	7
Species	2
Strains	2
Phases	3
Interactions	28
Species × strains	4
Species × phases	6
Strains × phases	6
Species × strains × phases	12
Total	35

The relation between these components and the division used in an analysis of variance is readily recognized.

The formula employed in obtaining χ^2 for the main effects, interactions, and total is as follows:

$$\chi^2=\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]+\frac{Sx}{Sy}\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]$$

in which

$N=$ total number of items classified

$Nu=$ number of items classified for the lowest category of the table

$x=$ number of items in any one category showing one or the other of the alternative phenomena

$y=$ number of items in any one category showing the other alternative phenomenon.

The detailed calculations for obtaining the total χ^2 for the data given in table 1 are given in table 2.

TABLE 1—*Univalent and lagging chromosomes in 100 cells of collections of Fragaria ovalis, varieties of* × *F. ananassa, and their F_1 hybrids during meiosis*

DETAILED DATA NOT GROUPED

Variate	First division (I)		Second division (II)		Total
	Metaphase	Telophase	Metaphase	Telophase	
Fragaria ovalis:	*Number*	*Number*	*Number*	*Number*	*Number*
37501	4	5	2	4	15
361477	6	4	3	1	14
36979	8	3	3	4	18
Total	18	12	8	9	47
F_1 hybrid:					
Dorsett × 37501	3	0	2	0	5
Gem × 361477	1	2	1	0	4
Fairfax × 36979	5	4	4	5	18
Total	9	6	7	5	27
× *F. ananassa:*					
Dorsett	2	1	0	1	4
Gem	5	2	2	1	10
Fairfax	1	0	3	2	6
Total	8	3	5	4	20

DATA GROUPED FOR SPECIES AND STRAINS

Species group	Row symbol	Sets[1] involving—			
		37501 and Dorsett	361477 and Gem	36979 and Fairfax	Total
Fragaria ovalis	r_1	15	14	18	47
F_1 hybrids	r_2	5	4	18	27
× *F. ananassa*	r_3	4	10	6	20
Total		24	28	42	94

[1] Column symbol for column 3=*a*, for column 4=*b*, and for column 5=*c*.

To complete the illustration of the method of partitioning total χ^2 into its components, the data grouped on the basis of one of the interactions need to be considered (see table 1). The calculations are given in table 2. The χ^2 for the data based on species and strains is 27.549. But this χ^2 for species and strains is composed of the χ^2 due to differences between species, the χ^2 due to differences between strains, and the χ^2 due to the interaction between species and strains. The χ^2 values for species and strains, also given in table 2, are 12.868 and 5.855. Subtracting these two values from the χ^2 value 27.549 gives the χ^2 (8.826) attributable to the interaction between species and strains.

TABLE 2.—*Details for calculating total and partitioned χ^2's for data in table 1*

Formula	Total χ^2	χ^2 for species and strains	χ^2 for species	χ^2 for strains
N	3, 600	3, 600	3, 600	3, 600
Nu	100	400	1, 200	1, 200
Sx	94	94	94	94
Sy	3, 506	3, 506	3, 506	3, 506
Sx^2	376	1, 262	3, 338	3, 124
$Sx.Nu$	9, 400	37, 600	112, 800	112, 800
$\frac{N}{Sx.Nu}$	0. 3829787234	0. 095744681	0. 031914894	0. 031914894
$\frac{N}{Sx.Nu}Sx^2$	144. 000000	120. 829787	106. 531916	99. 702129
$\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx$	50. 000000	26. 829787	12. 531916	5. 702129
$\frac{Sx}{Sy}$	. 026811181	. 026811181	. 026811181	. 026811181
$\frac{Sx}{Sy}\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx$	1. 340559	. 719338	. 335995	. 152881
$\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]+\frac{Sx}{Sy}\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]$	51. 340559	27. 549125	12. 867911	5. 855010
χ^2	51. 341	27. 549	12. 868	5. 855

Tables similar to the lower part of table 1 can be compiled for species and phases and for strains and phases. The methods of calculating the different χ^2's for the two resulting tables are identical with those used in calculating the various χ^2's for species and strains, and hence need not be given here. The χ^2 for the second-order interaction (species×strains×phases) may be obtained by subtracting the χ^2's for main effects and the χ^2's for the first-order interactions from the total χ^2, which, as previously calculated, is 51.341. All of these χ^2's are listed under Experimental Results (see table 4).

From table 2 and a probability table for χ^2, it can be seen that the odds are rather great against deviations as large as those noted between species occurring by chance. The same, to a lesser extent, is true of the differences between strains. This means that the interaction χ^2 (8.826) is not suitable for testing whether the deviations between the frequency distributions of table 1 for species in respect to strains can be attributed to chance (see Mather, *19*). Hence, it is necessary to calculate a heterogeneity χ^2.

The formula employed in calculating the heterogeneity χ^2 i sas follows:

$$\chi^2=W+W'$$

in which

$$W=\frac{Sx}{Sa}\left(\frac{a_1^2}{Sr_1}+\frac{a_2^2}{Sr_2}+\ .\ .\ .\ +\frac{a_n^2}{Sr_n}\right)+\frac{Sx}{Sb}\left(\frac{b_1^2}{Sr_1}+\frac{b_2^2}{Sr_2}+\ .\ .\ .\ +\frac{b_n^2}{Sr_n}\right)$$

$$+\ .\ .\ .\ +\frac{Sx}{Sz}\left(\frac{z_1^2}{Sr_1}+\frac{z_2^2}{Sr_2}+\ .\ .\ .\ +\frac{z_n^2}{Sr_n}\right)-Sx$$

TABLE 3.—*Percentage of Haemonchus contortus larvae recovered after outdoor exposure of the experimental pots in various locations*

MAXIMUM TEMPERATURES OF 26° TO 65° F. IN FIRST EXPOSURE PERIOD

Experiment No.	Date of infection of soil	Period of exposure	Mean maximum air temperature[1]	Total rainfall[2]	Average larvae in each of 4 controls	Proportion of larvae recovered[3] after exposure in—		
						Shade	Partial sun and shade	Sun
		Days	*° F.*	*Inches*	*Number*	*Percent*	*Percent*	*Percent*
15[4]	Jan. 31, 1940	6	41	0	6, 550	0	0	0
		13	45	.94		0	0	0
		20	44	1.69		0	0	0
10	Nov. 22, 1939	3	47	0	22, 100	0	.01	0
		6	48	0		0	0	0
		13	51	.65		0	0	0
		20	52	0		0	0	0
11[4]	Dec. 6, 1939	6	55	0	26, 650	0	0	0
		13	53	.48		0	0	0
		20	51	1.09		0	0	0
19	Mar. 27, 1940	3	63	0	3, 400	0	0	0
		6	64	.46		0	0	0
		13	62	2.50		0	0	0
		20	60	.69		0	0	0
13[4]	Jan. 3, 1940	6	30	.44	30, 550	0	0	0
		13	35	.94		0	0	0
		20	32	.85		0	0	0
12[4]	Dec. 20, 1939	6	42	.60	102, 800	0	0	0
		13	39	.20		0	0	0
		20	34	0		0	0	0
14[4]	Jan. 17, 1940	6	26	.85	2, 550	0	0	0
		13	27	.15		0	0	0
		20	33	0		0	0	0
9	Nov. 7, 1939	3	58	.94	80, 750	.006	.015	.002
		6	60	0		0	0	.001
		13	60	0		0	0	0
		20	56	0		0	0	0
17[4]	Feb. 28, 1940	6	47	1.22	108, 250	0	0	0
		13	47	0		0	0	0
		20	47	1.92		0	0	0
16[4]	Feb. 14, 1940	6	42	1.35	450	0	0	0
		13	42	1.36		0	0	0
		20	43	1.22		0	0	0
18[4]	Mar. 13, 1940	6	48	1.92	36, 000	0	0	0
		13	48	0		0	0	0
		20	52	.46		0	0	0
21	Apr. 24, 1940	3	60	2.46	35, 250	.003	.006	.01
		6	66	0		0	0	0
		13	70	.62		0	0	0
		20	73	0		0	0	0
20	Apr. 10, 1940	3	64	3.02	53, 200	0	0	0
		6	58	.17		0	0	0
		13	57	2.76		0	0	0
		20	60	.02		0	0	0
MAXIMUM TEMPERATURES OF 66° TO 84° F. IN FIRST EXPOSURE PERIOD								
7	Oct. 11, 1939	3	70	0	221, 200	0.01	0.002	0.0005
		6	68	0		.0005	0	.003
		13	69	.12		0	.03	.06
		20	68	.77		.005	.008	.02
24	June 19, 1940	3	78	0	3, 100	0	.03	.3
		6	81	0		0	0	1.8
		13	81	.76		.03	0	0
		20	81	1.50		0	.03	0
5	Sept. 22, 1939	3	83	0	425, 100	0	0	0
		6	81	.06		0	0	0
		13	74	3.88		0	.01	0
		20	77	0		0	.02	0
2	Aug. 30, 1939	3	85	0	12, 100	0	0	0
		6	86	2.46		0	0	0
		13	84	.53		.1	.03	.08
		20	83	.19		0	0	0
8	Oct. 25, 1939	3	71	.12	215, 850	4.7	26.9	2.0
		6	67	.77		.005	1.5	.9
		13	59	1.72		.04	.8	.04
		20	59	0		.02	.04	.02

TABLE 3.—*Percentage of Haemonchus contortus larvae recovered after outdoor exposure of the experimental pots in various locations*—Continued

MAXIMUM TEMPERATURES OF 66° TO 84° F. IN FIRST EXPOSURE PERIOD—Con.

Experiment No.	Date of infection of soil	Period of exposure	Mean maximum air temperature	Total rainfall	Average larvae in each of 4 controls	Proportion of larvae recovered after exposure in—		
						Shade	Partial sun and shade	Sun
		Days	° *F.*	*Inches*	*Number*	*Percent*	*Percent*	*Percent*
28	Sept. 11, 1940	3	70	.36	360,500	29.3	7.8	7.3
		6	72	.01		1.1	1.2	0
		13	77	0		.5	.3	0
		20	75	.88		.2	.9	0
30	Oct. 9, 1940	3	66	.44	194,400	2.9	4.6	1.4
		6	70	0		4.4	2.6	.001
		13	63	.47		.01	.004	0
		20	62	.15		0	0	0
22	May 8, 1940	3	73	.62	62,850	6.1	11.4	.002
		6	76	0		0	0	0
		13	78	.88		.3	3.3	0
		20	76	1.81		.2	0	0
4	Sept. 13, 1939	3	80	.72	483,900	12.5	15.6	.6
		6	81	0		.3	.9	.3
		13	81	0		.4	.2	.2
		20	78	3.94		.1	.03	0
29	Sept. 25, 1940	3	70	.88	238,750	26.5	14.5	5.1
		6	68	0		6.4	5.3	.005
		13	69	.84		6.3	8.1	.4
		20	69	.44		1.7	6.9	.1
6	Sept. 27, 1939	3	73	.92	355,450	34.7	41.2	24.9
		6	71	3.02		24.3	24.5	9.8
		13	73	0		21.7	18.4	8.2
		20	73	0		21.4	18.2	15.1
25	July 3, 1940	3	77	2.26	4,500	0	.02	0
		6	80	0		1.0	8.9	.4
		13	83	.26		.4	2.6	6.7
		20	86	0		1.3	.1	4.9
3	Sept. 8, 1939	3	84	2.99	287,000	32.1	26.6	3.0
		6	78	.19		36.1	26.6	5.7
		13	80	0		24.5	20.0	2.8
		20	80	.05		5.4	36.2	1.0
MAXIMUM TEMPERATURES OF 86° TO 93° F. IN FIRST EXPOSURE PERIOD								
26	July 17, 1940	3	88	0.26	11,700	0.2	0	0
		6	91	0		.1	.009	0
		13	93	1.15		9.4	0	0
		20	91	0		.6	0	0
23	June 5, 1940	3	87	.50	10,450	.6	.9	0
		6	86	0		6.3	.5	0
		13	85	0		12.8	0	.2
		20	84	0		11.9	0	0
27	July 31, 1940	3	86	.71	18,050	9.6	0	0
		6	86	0		21.4	.1	0
		13	87	0		.1	0	0
		20	85	1.12		4.1	.1	0
1	Aug. 16, 1939	3	93	1.57	24,500	0	0	0
		6	92	.06		5.4	.05	.004
		13	89	0		10.5	.02	0
		20	88	2.46		1.2	0	0

---

[1] Mean of maximum air temperatures in shade during entire indicated period of exposure.
[2] For method of computing, see text.
[3] Based on average number of larvae in control cultures.
[4] No larvae recovered from pots exposed 27 days; record omitted.

To facilitate the study of the data in table 3, they are summarized in table 4. The average percentage of recovery was computed by dividing the total of all larvae recovered in a given temperature and rainfall group, exposed for the same period and in the same location, by the total number of larvae in the corresponding controls.

W' is obtained by substituting the values of y for those of x in table 1 and solving the formula just given for W. For example, in substituting y for x, cell r_1, a_1 becomes 385 (400−15) instead of 15; r_2, a_2 becomes 395 (400−5); etc. Explanations of other symbols follow:

Sx = total number of the items showing one or the other of the alternative phenomena
Sy = total number of the items showing the alternative phenomenon
$Sa, Sb, \ldots, Sz$ = total number of items in the designated column
$a_1, a_2, \ldots, a_n$ = number of items in the designated category of column a
$b_1, b_2, \ldots, b_n$ = number of items in the designated category of column b
$z_1, z_2, \ldots, z_n$ = number of items in the designated category of the last column
$Sr_1, Sr_2, \ldots, Sr_n$ = total number of items in the designated row.

From the above formula it will be noted that a_1^2, b_1^2, . . ., z_1^2 are divided by Sr_1. Hence, machine calculation can be much simplified by expressing Sr_1 as a decimal fraction and locking the common multiplier in the machine. The same is true for the other calculations involving Sr. The detailed calculations for the lower part of table 1 follow:

$$Sx=94;\ Sa=24;\ Sb=28;\ Sc=42$$

$$S\left(\frac{a^2}{Sr}\right)=6.513160;\ S\left(\frac{b^2}{Sr}\right)=9.762806;\ S\left(\frac{c^2}{Sr}\right)=20.693617$$

$$\frac{Sx}{Sa}=3.916667;\ \frac{Sx}{Sb}=3.357143;\ \frac{Sx}{Sc}=2.238095$$

$$W=10.599295;\ W'=0.230365;\ \chi^2=10.829660$$

In such problems as those illustrated in which the main effects differ materially, the heterogeneity χ^2 is preferred to the interaction χ^2 obtained by partitioning the total χ^2 into its components. However, in many problems it is not necessary to calculate the heterogeneity χ^2, which involves much more work.

EXPERIMENTAL RESULTS

It can be seen from table 1, without detailed statistical analysis, that meiosis is essentially normal as regards synapsis. This fact is readily appreciated when it is recalled that the figures in the individual categories represent the number of cells among 100 examined that showed at least 1 univalent chromosome. Hence, asynapsis is not frequent enough to interfere materially with the obtaining of double crosses and advanced and backcross populations. It follows, then, that asynapsis is not a factor in breeding new varieties of strawberries by the hybridization method in which × *Fragaria ananassa* and *F. ovalis* are used as parents.

The occurrence of associations of more than two chromosomes to form multivalents during meiosis was so infrequent as not to merit tabulation. It may be concluded, therefore, that this phenomenon also presents no problem in breeding strawberries by the hybridization method in which × *Fragaria ananassa* and *F. ovalis* are used as the parents.

The third major problem is that of determining the type of conjugation occurring during meiosis of the F_1 hybrids. If autosyndesis is taking place during meiosis of the F_1 hybrids between × *Fragaria ananassa* and *F. ovalis*, then the × *F. ananassa* chromosomes are pairing with × *F. ananassa* chromosomes and the *F. ovalis* chromosomes with *F. ovalis* chromosomes. By using the previously given symbols for the chromosome complements of × *F. ananassa* and *F. ovalis*, autosyndesis in the F_1 hybrids may be illustrated as follows:

× *F. ananassa:*
$Acv_1; Acv_2; Acv_3; Acv_4$
$Acv_1; Acv_2; Acv_3; Acv_4$
F. ovalis:
$Ao_1; Ao_2; Ao_3; Ao_4$
$Ao_1; Ao_2; Ao_3; Ao_4$
F_1 hybrid:
$Acv_1; Acv_2; Ao_1; Ao_2$
$Acv_3; Acv_4; Ao_3; Ao_4$

From the above it is clear that, if autosyndesis is occurring, all of the F_2 populations would be composed of four complete sets of × *F. ananassa* chromosomes and four complete sets of *F. ovalis* chromosomes. Hence, if × *F. ananassa* chromosomes do not conjugate with *F. ovalis* chromosomes, there is no opportunity for segregation of the genes differentiating the interspecific characters, that is, those genes differentiating the characters by which the species differ.

Similarly, allosyndesis in an F_1 hybrid may be illustrated as follows:

$Acv_1; Acv_2; Acv_3; Acv_4$
$Ao_1; Ao_2; Ao_3; Ao_4$

From this illustration it can be seen that the maximum opportunity for segregation of the genes differentiating the interspecific characters would occur during meiosis.

One form of a combination of the two types of conjugation in an F_1 hybrid may be illustrated as follows:

$Acv_1; Ao_1; Acv_3; Acv_4$
$Acv_2; Ao_2; Ao_3; Ao_4$

From this illustration it can be seen that in some cases × *Fragaria ananassa* chromosomes are paired with × *F. ananassa* chromosomes; in other cases they are paired with *F. ovalis* chromosomes. It follows that in some instances *F. ovalis* chromosomes are paired with *F. ovalis* chromosomes; in others they are paired with × *F. ananassa* chromosomes. Hence, the opportunity for segregation of the genes differentiating the interspecific characters is intermediate between those of autosyndesis and allosyndesis.

From these considerations it is evident that the type of pairing of the chromosomes (autosyndesis, allosyndesis, or a combination of the two) materially influences the segregation of the genes that differentiate the interspecific characters. The commonest method of chromosome pairing of polyploid organisms is allosyndesis. With this fact in mind, Wright (*28, p. 45*) has shown that—

> a random-bred stock derived from *n* inbred families will have $\frac{1}{n}$th less superiority over its inbred ancestry than the first cross or a random-bred stock from which the inbred families might have been derived without selection.

Kiesselbach (*12*), working with plants, reached a similar conclusion.

Wright has developed formulas for use when the effects of the genes differentiating the characters under consideration are geometrically cumulative. For these formulas and their application, the reader is referred to Powers (*22*) and Powers and Lyon (*23*). These formulas as well as those based on the assumption that the effects of the genes are arithmetically cumulative assume that allosyndesis is occurring during meiosis of the F_1 hybrid. Thus it is clear that, if the means of the interspecific characters can be predicted by the use of Wright's formulas, allosyndesis must be occurring during meiosis of the microsporocytes and the megasporocytes at least as regards the chromosomes containing the genes which differentiate the characters under consideration. On the other hand, if autosyndesis is the rule, segregation of the genes differentiating the interspecific characters would be materially limited and the means of the characters for the F_2 populations should closely approximate the means of the same characters for the F_1 populations.

In making these tests the studies will be more conclusive if the magnitude of the effects of the genes differentiating the two species is quite large as regards the character under consideration and if the characters are based on absolute rather than observational measurements. The characters which meet these specifications are plant height measured in centimeters, number of days from May 1 to first bloom, and number of days from first bloom to first fruit ripe. Although based only on observational grades, the degree of winter injury was very marked and, therefore, this character also was included in the study. The degree of winter injury increases from grade 1 to grade 5.

The data are presented in table 3. The theoretical means listed are those calculated on the assumption that the effects of the genes differentiating the respective characters are arithmetically cumulative. The fit between the theoretical means calculated on the assumption that the effects of the genes are geometrically cumulative and the obtained means (with the exception of the means for grades of winter injury) was definitely poorer than the fit between the means calculated on the assumption that the effects of the genes are arithmetically cumulative and the obtained means. Since such is the case, there would not be much point in listing the theoretical geometric means. In respect to winter injury the geometric mean for the backcross to Fairfax fitted the obtained mean better than did the arithmetic mean, but for the F_2 population the reverse was true.

TABLE 3.—*The obtained and theoretical arithmetic means and their standard errors for different characters of the cross Fairfax × Fragaria ovalis (36979)*

Variate	Winter injury		Plant height		Period from May 1 to first bloom		Period from first bloom to first fruit ripe	
	Obtained	Theoretical	Obtained	Theoretical	Obtained	Theoretical	Obtained	Theoretical
Fragaria ovalis **(36979):**	*Grade*	*Grade*	*Centimeters*	*Centimeters*	*Days*	*Days*	*Days*	*Days*
Asexual	1.00±0.170		11.3±0.597		14.2±0.696		46.0±0.943	
Selfed (S_1)	1.06± .124		8.8± .554		14.5± .477		49.1± .605	
Hybrid populations:								
Backcross [(Fairfax×36979)×36979]	1.07± .141	1.16±0.112	16.3± .633	14.5±0.448	14.3± .517	15.3±0.371	44.6± .702	43.8±0.510
F_1 (Fairfax×36979)	1.32± .146		17.7± .668		16.3± .260		40.8± .389	
F_2 (Fairfax×36979)	2.03± .107	2.19± .111	13.1± .345	14.1± .475	22.3± .472	19.7± .870	40.5± .330	40.2± .591
Backcross [Fairfax×(Fairfax×36979)]	2.79± .132	3.21± .110	14.2± .395	13.7± .501	25.2± .610	24.1±1.173	36.3± .515	36.9± .662
×*F. ananassa* **(Fairfax):**								
Selfed (S_1)	4.65± .169		9.6± .748		31.8±2.332		33.0±1.265	
Asexual	5.10± .165							

The means for winter injury and for days from May 1 to first bloom ranged from that of *Fragaria ovalis* asexually propagated to that of Fairfax asexually propagated or self-pollinated; for days from first bloom to first fruit ripe the means ranged from that of the progeny of Fairfax self-pollinated to that of the progeny of *F. ovalis* self-pollinated. In plant height, for which the F_1 hybrid shows decided heterosis, the means ranged from that of the progeny obtained by self-pollinating *F. ovalis* to that of the F_1 hybrid. These data are in accord with what would be expected if allosyndesis was occurring during meiosis of the F_1 hybrid. It will be recalled that in case autosyndesis was the common mode of behavior the mean of the F_2 population would be expected to be similar in magnitude to that of the F_1 hybrid. As may be seen from the data in table 3, such definitely was not the case as regards winter injury, plant height, and days from May 1 to first bloom. As regards days from first bloom to first fruit ripe, a comparison of the means for the F_1 hybrid and the F_2 population does not indicate whether allosyndesis or autosyndesis is the rule. The reason for this is that the theoretical arithmetic mean based on allosyndesis is so close to that of the F_1 hybrid that any differences noted can logically be attributed to chance deviations. Probably the most convincing evidence that allosyndesis rather than autosyndesis or a combination of allosyndesis and autosyndesis is the rule during meiosis of the F_1 hybrid is that in 10 cases out of a possible 12 (see Tippett *27, p. 54*) the differences between the obtained and theoretical means can logically be attributed to chance deviations.

In summing up it may be said that the evidence, which is rather conclusive, is preponderantly in favor of allosyndesis as the type of conjugation that occurs during meiosis of the F_1 hybrids, and it appears certain that any deviation from allosyndesis is not of sufficient importance to interfere materially with obtaining the objectives of a breeding program in which × *Fragaria ananassa* (variety Fairfax) and *F. ovalis* (collection 36979) are the parents.

From the foregoing it can be seen that the major problems which the experiment was designed to answer have been solved. However, the data furnish additional information which, though of only minor importance to the breeding program at Cheyenne, may be of interest to other investigators working with the cytogenetics of *Fragaria*.

The χ^2 values for testing goodness of fit between the theoretical based on the supposition that there are no differences between species, between strains, or between phases, together with those χ^2 values for testing whether the interactions are statistically significant, are given in table 4. From these data it seems probable that differences exist between species, between strains, and between phases. However, the differences between strains are not so well established statistically as the differences between species or between phases. The only interaction approaching statistical significance is that between species and strains. The heterogeneity χ^2 for this interaction is 10.830, which is highly significant.

The meaning of these differences can best be found by examining the data in table 1. The differences between species there shown were due to the fact that failure of chromosome pairing was somewhat greater in the native Rocky Mountain strawberry than in the cultivated strawberry. The possible differences between strains were due to the fact that the failure of chromosome pairing was at least partially dominant in the F_1 hybrid of Fairfax × 36979 and at least partially recessive in the F_1 hybrids of Dorsett × 37501 and Gem × 361477. The differences between phases were due to the fact that the number of cells showing irregularities was greater for the metaphase of the first division than for the telophase of that division and either stage of the second division. The explanation for this may be the same as that found by Powers (*21*) in similar studies with *Triticum aestivum*.

TABLE 4.—χ^2 *values for the different components*

Source of variation	Degrees of freedom	χ^2
Main effects:		
Species	2	12.868
Strains	2	5.855
Phases	3	7.909
Interactions:		
Species × strains	4	8.826
Species × phases	6	3.037
Strains × phases	6	4.020
Species × strains × phases	12	8.826
Total	35	51.341

Environment is a factor that must be considered in evaluating the importance of the frequency of failure of chromosome pairing to a crop-improvement program. The investigations of Stow (*26*), Heilborn (*9*), Randolph (*24*), and Myers and Powers (*20*) definitely show that the differences in environment encountered under field conditions have diverse effects on synapsis. So it must be concluded that, even though asynapsis influences the breeding program very little under the environmental conditions existing at Cheyenne, Wyo., it may be of major importance under the environmental conditions of other locations. For this reason, selections made at Cheyenne from material resulting from crossing ×*Fragaria ananassa* and *F. ovalis* may fail when grown in other localities.

SUMMARY

Cytological data, taken from three cultivated varieties of strawberry (×*Fragaria ananassa*), three collections of the native Rocky Mountain strawberry (*F. ovalis*), and three F_1 interspecific hybrids involving these collections and varieties, are reported.

New formulas for χ^2 are given, and their application is illustrated. These formulas reduce the labor involved in calculating χ^2 when the number of categories of the table used in the calculations is large.

The breeding data show rather conclusively that meiotic instability is at most a minor problem under the environmental conditions prevailing at Cheyenne, Wyo.

The segregation of the genes differentiating certain characters, as indicated by the means of these characters for different populations, furnishes rather convincing evidence that allosyndesis is the rule during meiosis of the F_1 hybrids.

So far as meiotic irregularities and the method of pairing of the chromosomes are concerned, there does not seem to be any reason why the economically important characters of ×*Fragaria ananassa* and *F. ovalis* cannot be recombined into a single variety adapted to production under the environmental conditions encountered at Cheyenne, Wyo.

LITERATURE CITED

(1) Belling, J.
1926. THE IRON-ACETOCARMINE METHOD OF FIXING AND STAINING CHROMOSOMES. Biol. Bul. 50: 160–162, illus.

(2) Darrow, G. M.
1937. STRAWBERRY IMPROVEMENT. U. S. Dept. Agr. Yearbook 1937: 445–495, illus.

(3) Dogadkina, N. A.
1941. A CONTRIBUTION TO THE QUESTION OF GENOME RELATIONS IN SOME SPECIES OF FRAGARIA. Acad. des Sci. U. R. S. S. Compt. Rend. (Dok.) 30: 166–168, illus.

(4) East, E. M.
1928. HEREDITY IN THE GENUS FRAGARIA WITH SPECIAL REFERENCE TO THE FALSE HYBRIDS OF MILLARDET. Verhandl. V. Internatl. Kong. f. Vererbungswiss. (International Congress of Genetics), Berlin, 1927, 1: [625]–630.

(5) ———
1930. THE ORIGIN OF THE PLANTS OF MATERNAL TYPE WHICH OCCUR IN CONNECTION WITH INTERSPECIFIC HYBRIDIZATIONS. Natl. Acad. Sci. Proc. 16: [377]–380.

(6) Fedorova, N.
1934. POLYPLOID INTER-SPECIFIC HYBRIDS IN THE GENUS FRAGARIA. Genetica 16: [524]–541, illus.

(7) Fisher, R. A.
1934. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London..

(8) Georgeson, C. C.
1923. PRODUCTION OF IMPROVED HARDY STRAWBERRIES FOR ALASKA. Alaska Agr. Expt. Stas. Bul. 4, 13 pp., illus.

(9) Heilborn, O.
1928. ZYTOLOGISCHE STUDIEN ÜBER POLLENSTERILITÄT VON APFELSORTEN. Svensk Bot. Tidskr. 22: [185]–199, illus.

(10) Hildreth, A. C., and Powers, L.
1941. THE ROCKY MOUNTAIN STRAWBERRY AS A SOURCE OF HARDINESS. Amer. Soc. Hort. Sci. Proc. 38: 410–412.

(11) Ichijima, K.
1930. STUDIES ON THE GENETICS OF FRAGARIA. Ztschr. f. Induktive Abstam. u. Vererbungslehre 55: [300]–347, illus.

(12) Kiesselbach, T. A.
1935. THE POSSIBILITIES OF MODERN CORN BREEDING. World's Grain Exhib. and Conf. Proc. (1933) 2: 92–112.

(13) Kihara, H.
1930. KARYOLOGISCHE STUDIEN AN FRAGARIA MIT BESONDERER BERÜCKSICHTIGUNG DER GESCHLECHTSCHROMOSOMEN. Cytologia 1: 345–357, illus.

(14) Kuz'min, A. IA.
1938. ONEKOTORYKH RESUL'TATAKH MEZHVIDOVOĬ GIBRIDIZAT͡SII U RIBES, RUBUS I FRAGARIA. (SOME RESULTS OF INTERSPECIFIC HYBRIDIZATION IN RIBES, RUBUS AND FRAGARIA.) Akad. Nauk S. S. S. R. Izv. (Acad. des Sci. U. R. S. S. Bul.): [681]–692, illus. [In Russian. English summary, p. 692.]

(15) Lilienfeld, F. A.
1933. KARYOLOGISCHE UND GENETISCHE STUDIEN AN FRAGARIA. I. EIN TETRAPLOIDER FERTILER BASTARD ZWISCHEN F. NIPPONICA (N=7) UND F. ELATIOR (N=21). Japan Jour. Bot. 6: [425]–458, illus.

(16) ———
1936. KARYOLOGISCHE UND GENETISCHE STUDIEN AN FRAGARIA II. IST FRAGARIA ELATIOR EINE AUTOPOLYPLOIDE PFLANZE? Jap. Jour. Bot. 8: [119]–149, illus.

(17) Longley, A. E.
1926. CHROMOSOMES AND THEIR SIGNIFICANCE IN STRAWBERRY CLASSIFICATION. Jour. Agr. Res. 32: 559–568, illus.

(18) Mangelsdorf, A. J., and East, E. M.
1927. STUDIES ON THE GENETICS OF FRAGARIA. Genetics 12: 307–339, illus.

(19) Mather, K.
[1938]. THE MEASUREMENT OF LINKAGE IN HEREDITY. 132 pp., illus. London.

(20) Myers, W. M., and Powers, L.
1938. MEIOTIC INSTABILITY AS AN INHERITED CHARACTER IN VARIETIES OF TRITICUM AESTIVUM. Jour. Agr. Res. 56: 441–452.

(21) Powers, L.
1932. CYTOLOGIC AND GENETIC STUDIES OF VARIABILITY OF STRAINS OF WHEAT DERIVED FROM INTERSPECIFIC CROSSES. Jour. Agr. Res. 44: 797–831, illus.

(22) ———
1939. STUDIES ON THE NATURE OF THE INTERACTIONS OF THE GENES DIFFERENTIATING QUANTITATIVE CHARACTERS IN A CROSS BETWEEN LYCOPERSICON ESCULENTUM AND L. PIMPINELLIFOLIUM. Jour. Genet. 39: [139]–170.

(23) ——— and Lyon, C. B.
1941. INHERITANCE STUDIES ON DURATION OF DEVELOPMENTAL STAGES IN CROSSES WITHIN THE GENUS LYCOPERSICON. Jour. Agr. Res. 63: 129–148.

(24) Randolph, L. F.
1932. SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE. Natl. Acad. Sci. Proc. 18: 222–229.

(25) Rozanova, M. A.
1938. MEZHVIDOVAI͡A GIBRIDIZATSII͡A V PREDELE RODOV RUBUS I FRAGARIA V SVIAZI S VOPROSAMI FORMOOBRAZOVANII͡A. (HYBRIDIZATION WITHIN THE GENERA RUBUS AND FRAGARIA AS RELATED TO PROBLEMS OF FORM-GENESIS). Akad. Nauk S. S. S. R. Izv. (Acad. des Sci. U. R. S. S. Bul.): [667]-679, illus. [In Russian. English summary, pp. 677–679.]

(26) Stow, I.
1926. A CYTOLOGICAL STUDY ON THE POLLEN STERILITY IN SOLANUM TUBEROSUM L. Imp. Acad. Japan, Proc. 2: 426–430, illus.

(27) Tippett, L. H. C.
1931. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 pp., illus. London.

(28) Wright, S.
1922. THE EFFECTS OF INBREEDING AND CROSSBREEDING ON GUINEA PIGS. Pt. III. U. S. Dept. Agr. Bul. 1121, 61 pp., illus.

(29) Yarnell, S. H.
1931. GENETIC AND CYTOLOGICAL STUDIES ON FRAGARIA. Genetics 16: 422–454, illus.

(30) ———
1931. A STUDY OF CERTAIN POLYPLOID AND ANEUPLOID FORMS IN FRAGARIA. Genetics 16: 455–489, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1944

(17) LONGLEY, A. E.
1926. CHROMOSOMES AND THEIR SIGNIFICANCE IN STRAWBERRY CLASSIFICATION. Jour. Agr. Res. 32: 559–568, illus.

(18) MANGELSDORF, A. J., and EAST, E. M.
1927. STUDIES ON THE GENETICS OF FRAGARIA. Genetics 12: 307–339, illus.

(19) MATHER, K.
[1938]. THE MEASUREMENT OF LINKAGE IN HEREDITY. 132 pp., illus. London.

(20) MYERS, W. M., and POWERS, L.
1938. MEIOTIC INSTABILITY AS AN INHERITED CHARACTER IN VARIETIES OF TRITICUM AESTIVUM. Jour. Agr. Res. 56: 441–452.

(21) POWERS, L.
1932. CYTOLOGIC AND GENETIC STUDIES OF VARIABILITY OF STRAINS OF WHEAT DERIVED FROM INTERSPECIFIC CROSSES. Jour. Agr. Res. 44: 797–831, illus.

(22) ———
1939. STUDIES ON THE NATURE OF THE INTERACTIONS OF THE GENES DIFFERENTIATING QUANTITATIVE CHARACTERS IN A CROSS BETWEEN LYCOPERSICON ESCULENTUM AND L. PIMPINELLIFOLIUM. Jour. Genet. 39: [139]–170.

(23) ——— and LYON, C. B.
1941. INHERITANCE STUDIES ON DURATION OF DEVELOPMENTAL STAGES IN CROSSES WITHIN THE GENUS LYCOPERSICON. Jour. Agr. Res. 63: 129–148.

(24) RANDOLPH, L. F.
1932. SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE. Natl. Acad. Sci. Proc. 18: 222–229.

(25) ROZANOVA, M. A.
1938. MEZHVIDOVAÎÂ GIBRIDIZATSIÎÂ V PREDELE RODOV RUBUS I FRAGARIA V SVIAZI S VOPROSAMI FORMOOBRAZOVANÎÂ. (HYBRIDIZATION WITHIN THE GENERA RUBUS AND FRAGARIA AS RELATED TO PROBLEMS OF FORM-GENESIS). Akad. Nauk S. S. S. R. Izv. (Acad. des Sci. U. R. S. S. Bul.): [667]–679, illus. [In Russian. English summary, pp. 677–679.]

(26) STOW, I.
1926. A CYTOLOGICAL STUDY ON THE POLLEN STERILITY IN SOLANUM TUBEROSUM L. Imp. Acad. Japan, Proc. 2: 426–430, illus.

(27) TIPPETT, L. H. C.
1931. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 pp., illus. London.

(28) WRIGHT, S.
1922. THE EFFECTS OF INBREEDING AND CROSSBREEDING ON GUINEA PIGS. Pt. III. U. S. Dept. Agr. Bul. 1121, 61 pp., illus.

(29) YARNELL, S. H.
1931. GENETIC AND CYTOLOGICAL STUDIES ON FRAGARIA. Genetics 16: 422–454, illus.

(30) ———
1931. A STUDY OF CERTAIN POLYPLOID AND ANEUPLOID FORMS IN FRAGARIA. Genetics 16: 455–489, illus.

o

U. S. GOVERNMENT PRINTING OFFICE: 1944

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 69 WASHINGTON, D. C., DECEMBER 15, 1944 No. 12

SOME HOST-PARASITE RELATIONS IN THE BLACK ROOT ROT OF APPLE TREES[1]

By J. S. COOLEY

Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

In investigating black root rot, the writer began with studies of the causal fungus (*Xylaria mali* Fromme), but as the work progressed to a study of conditions affecting infection the host-parasite relations when the hosts were growing under normal and abnormal conditions assumed greater importance. In this paper are reported the results of inoculating apple seedlings (*Malus pumila* Mill.) with *X. mali* at monthly intervals; the effects of defoliation and other maltreatments on susceptibility of apple are also reported.

METHODS

Unless otherwise stated, the method of inoculation consisted in uncovering root tissue below the collar of the tree, wounding by cutting or scraping the bark, placing a piece of inoculum in the wound, and finally replacing the soil. At first, inoculations were made through flaps cut entirely through the bark, but later experiments showed that deep wounding was not necessary or desirable. Beginning in 1934, the wounds were made by merely scraping the bark of the root with a trowel. The inoculum consisted of heat-sterilized apple twigs about 2 inches long, on which the pathogen had been grown for at least 2 months. Some of the trees used were grown for an experimental period of 2 to 6 years in nurseries at the Arlington Experiment Farm, Arlington, Va., or at the Plant Industry Station, Beltsville, Md. Since infection did not occur on uninoculated trees or even on the uninoculated part of inoculated roots, it was considered unnecessary to leave part of each plot uninoculated as a check on infection.

Each year, after killing frosts had occurred in the autumn, the soil at the point of inoculation was removed, the number of infections counted, and the length and the breadth of the lesions measured. As in an earlier report on black root rot (*1*),[2] the sizes of lesions were computed in the following manner. For each lesion the length was multiplied by the breadth to determine the approximate area. The square root of the resultant value was then derived, giving a linear value equal to one side of a square having an area approximating that of the lesion. The values recorded in the tables are averages of such relative linear functions for all lesions in each designated plot.

---

[1] Received for publication May 5, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 457.

EFFECT OF INOCULATING SEEDLINGS AT MONTHLY INTERVALS

In the earlier inoculation experiments irregular and often negative results had been obtained. It was thought that such results might be due to inoculating at the wrong time of the year. For this reason a series of inoculations made at monthly intervals was started in the spring of 1932 and continued until November 1935. At the beginning of the experiment the trees were seedlings growing their second season in nursery rows at the Arlington Experiment Farm. Twenty to thirty trees were inoculated at as nearly the middle of each month as was practicable.

In 1932 inoculations were made with culture 3, but all subsequent inoculations were made with culture 57. The trees were too small in 1932 for crowding to be serious or moisture to be deficient, but this was not the case in later years. No infections resulted from the use of culture 3. The results with culture 57 for 3 consecutive years are recorded graphically in figure 1.

The results of the 1932 inoculations, all of which were negative, appeared at first to be aberrant, but there were certain conditions which at least partly account for these results. The trees were young, vigorous, and not crowding one another, and the season was favorable for growth. It is significant that, when the trees inoculated in 1932 were examined in August of that year, there were small lesions 3 to 5 mm. in diameter on some of the trees inoculated in July but none on those inoculated in any other month. By October or November the small lesions observed in August had healed over, leaving only scars; they were then recorded as not infected. The effect of host condition on infection is further shown by another experiment in which some bearing trees in an abandoned orchard at Arlington Experiment Farm were inoculated in August 1932 in a parallel series and with the same isolate (No. 3) used for the inoculations of yearling nursery trees. The inoculations of these old trees resulted in a fair percentage of infections, and lesions as long as 110 mm. were produced.

In 1934 and 1935, which were the fourth and fifth growing seasons for the young trees, conditions were apparently much more favorable for infection than in previous years. In general, a much higher percentage of infection resulted from the June and July inoculations than from those made at any other time. The percentages of infection for 1933 to 1935 rose in June, reached their peak in July, and declined to less than 10 percent by September. Susceptibility at any particular date may differ from season to season. For example, infection for August 1934 was 57 percent, but for August 1935 it was only 9 percent. The July infections were 83 and 88 percent, respectively, in the 2 years. These records indicate that conditions were favorable for the extension of the infection period into August in 1934 but not in 1935.

Since *Xylaria* has a fairly high optimum temperature, about 25° C. (77° F.), when growing in artificial culture, it is possible that the increase in infection in June and the decline in August may have been due to soil temperatures favorable for the fungus in June and July and unfavorable during the rest of the summer. However, infection of dug apple seedlings has been obtained by the writer (*1*) at a temperature as low as 15° C.

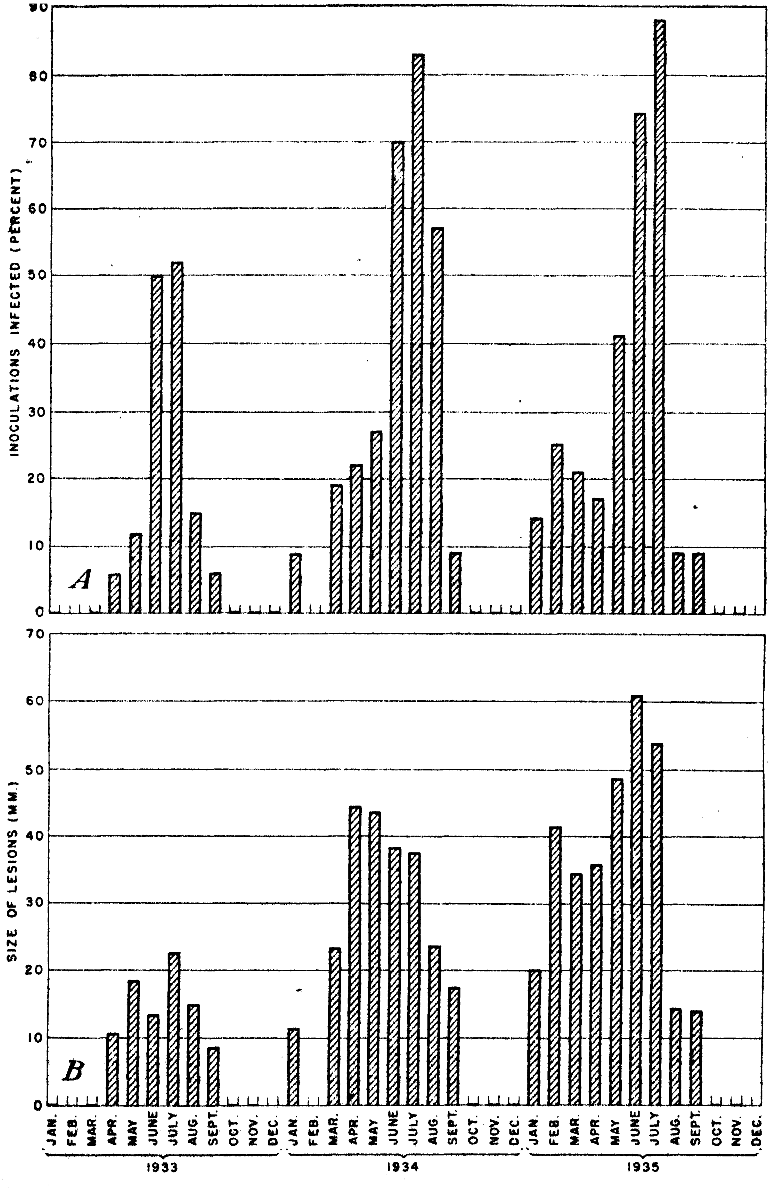


Figure 1.—Infection results from monthly inoculations of young apple trees with *Xylaria mali*. *A*, Average percentage of inoculations resulting in infections on the same trees in the months indicated in 3 consecutive years. *B*, Average size of lesions represented in *A*. The size is expressed as a side of a square having approximately the area of the lesion.

The infections resulting from the winter and early-spring inoculations, when the soil was too cold for *Xylaria* to grow, may have been due to the fungus remaining viable and commencing growth when temperature conditions became favorable.

Soil-temperature records were not kept at Arlington Experiment Farm while these experiments were in progress, but air-temperature records were available for 1933, 1934, and 1935 (fig. 2). In 1924 Olmstead (*5*) had obtained records of both air and soil temperatures (fig. 3). These curves, which follow each other closely, rose gradually to a maximum about August 1 and then gradually declined. Olmstead's air-temperature curve is similar to those for 1933 to 1935 (fig. 2). If the soil temperatures followed the air temperatures in these years as they did in 1924, they should have been sufficiently high for infection in August and September, when infection was relatively slight. This suggests that some factor other than temperature change may have been responsible for the little infection found.

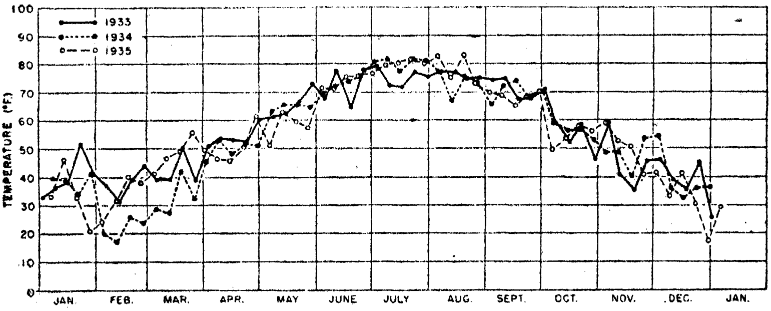


FIGURE 2.—Average weekly air temperature at Arlington Experiment Farm for 1933, 1934, and 1935.

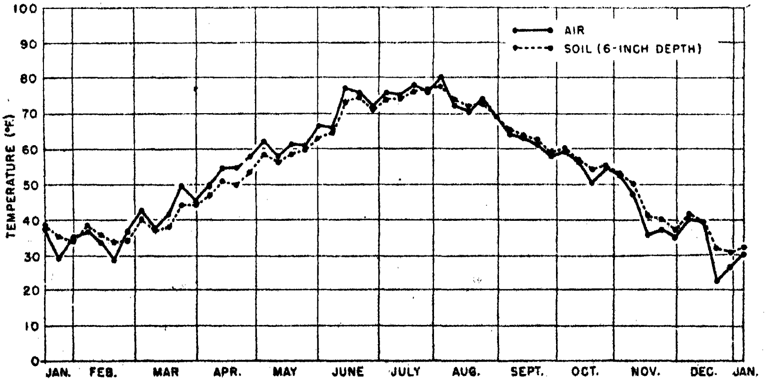


FIGURE 3.—Average weekly air and soil temperatures at Arlington Experiment Farm for 1924. (Adapted from data of Olmstead (*5*).)

The weather record for Arlington Experiment Farm for 1934 shows in average air temperature for July and August of 77° F. and 73.8°, respectively, and for 1935, 80.2° and 77.9°. The rainfall for July and August 1934 was 1.79 and 5.07 inches, respectively, and for July and August 1935 it was 2.77 and 2.25 inches.

The weather conditions that predispose the host to the disease and that favor the development of the pathogen are too complex for proper evaluation. The difference in the percentage of infection in August 1934 and 1935 is probably associated with environmental conditions, but more information than the usual weather records would be necessary to explain the difference in infection trends. The factors contributing to high susceptibility in midsummer are discussed later in the paper.

EFFECT OF SEASONAL MALTREATMENTS ON SUSCEPTIBILITY OF HOST

Periodic inoculations have shown that at certain times of the year apple is very susceptible to infection by *Xylaria* and at other times it is much less so. To study further the effect of the physiological condition of the host on susceptibility, experiments designed to modify the translocation and storage processes of the host by various periodic maltreatments were carried out during the summer of 1936. The experiments were designed to determine whether interruption or modification of growth processes at different growth periods would influence resistance of roots to black root rot. Five plots of 20 to 30 trees each for each treatment and at each treatment period were selected at random in a block of 4-year-old apple seedlings. Plots were treated during the summer months as follows: (1) Trunks were cut more than half through, and the tops were bent over; (2) roots were severely pruned by inserting a spade 12 inches deep and 15 inches from the tree; (3) most of the foliage-bearing branches, including a major part of the previous year's growth, were removed; (4) a ring of bark about 6 mm. wide was removed from the trunk just above the soil line; (5) no maltreatments were given.

The maltreatments were begun in April about the time of the swelling of the leaf buds and were repeated at monthly intervals until the middle of August, after which previous experiments had indicated that inoculations were usually unsuccessful. In mid-July roots were inoculated on trees treated up to that time and on untreated trees, and the remaining trees received both maltreatment and inoculation in mid-August. The results of this experiment are shown in table 1.

TABLE 1.—*Effect of maltreatments in different months on infection by Xylaria mali in 1936*

[20 to 30 trees inoculated in each treatment on each inoculation date; results taken in November]

Treatment	Trees infected when inoculated on July 14 but treated on indicated date					Trees infected when treated and inoculated on August 14
	April 8	May 8	June 5	July 8	July 14	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None (check)	76	76	76	76	76	55
Trunk cut and bent	91	90	96	85	93	81
Roots pruned	85	50	86	----------	----------	----------
Trunk ringed	100	100	94	89	87	----------
Branches summer-pruned	----------	75	91	84	84	90

Table 1 shows in general slightly less infection on the unwounded trees than on those receiving the maltreatments. These differences were usually more pronounced when maltreatments were given in June and inoculations were made in July than when maltreatments were given in July and inoculations made in July. The differences, however, between the untreated trees and maltreated trees were not as great as might have been expected from such harsh treatment. For the trees receiving no maltreatments the average size of lesion for July inoculation was about 35 mm.; for the maltreated trees the average sizes for the different periods of treatments ran from 40 to 45 mm.

Since root pruning was difficult to carry out in dry gravelly soil it was discontinued after June. Root pruning in the main showed the same tendency to increase infection as the other maltreatments. However, the number of infections was low on the trees root-pruned in May.

Ringing gave more irregular results than any other treatment. If the girdle was partially closed by callus and the bark was not directly killed by girdling, very large lesions resulted from inoculations made 3 to 4 inches below the girdle. However, if the girdle was not partially closed by callus, the bark usually died below the girdle and the dying extended to the inoculation or below it. In such cases the black root rot lesions were usually small but distinguishable from the adjacent necrotic tissue by their intense black color. Apparently, as general necrosis approached the inoculation point the advance of the fungus was impeded or stopped. Possibly with death of the host tissue some substance toxic to the fungus developed. However, observations by the writer have shown that when the fungus attacks and kills living roots it may remain alive long after the death of the host. The general trend of the results of maltreatments was a slight increase in the percentage of trees infected and in the size of lesions as compared with trees not maltreated.

EFFECT OF DEFOLIATION ON SUSCEPTIBILITY OF HOST

The experiment just described showed that summer pruning involving considerable loss of leaves increased the susceptibility of the host to *Xylaria*. Defoliation by leaf removal modifies food synthesis and associated processes with less mutilation than defoliation by summer pruning. The drastic effect of defoliation on the periodic interdependence of root and top would be expected to affect the susceptibility of the roots to this disease.

Preliminary experiments with defoliation by leaf removal were begun in the summer of 1934 on 2-year-old apple trees in a nursery at Arlington Experiment Farm. The results indicated a possible relation between defoliation and susceptibility. In these preliminary experiments the trees defoliated in August had a higher percentage of infection and larger lesions than the nondefoliated check trees.

In 1937 an experiment was begun to study the effect on root susceptibility of defoliating trees at different times throughout the growing season. Three-year-old Stayman Winesap trees worked on seedling roots were set 2 feet apart in rows 4 feet wide and divided into plots of 20. Randomized plots were defoliated at semimonthly intervals from May 3 to October 18, that is, from the time when young shoots were 3 to 4 inches long until the leaves had been frozen. Since previous experience had shown July to be the most favorable period for infection by *Xylaria*, the first inoculation of 1937 was made on July 15 on the previously defoliated plots and on one nondefoliated (check) plot. For the remainder of the season inoculations were made at the time of each semimonthly defoliation; nondefoliated (check) plots were inoculated at the same time. In order to allow a reasonable period for root rot development, recording of infections was delayed until April 1938.

In 1938 the treatments were repeated on all the plots on essentially the same dates as in 1937; inoculations were made on the opposite side of the trees, and the record of infections was taken the following spring. Defoliations were not repeated in 1939; but all trees were inoculated in mid-July, and the results were recorded at the end of the growing season. The results of the experiment are recorded in table 2.

TABLE 2.—*Effect of defoliation on susceptibility of Stayman Winesap apple trees to Xylaria mali*

Date of first defoliation	Date of second defoliation	Date of inoculation	Trees infected			Size of lesion
			Total infection	Cortical infection	Deep infection	
			Percent	*Percent*	*Percent*	*Mm.*
May 3, 1937	----	July 15, 1937	33	19	14	16
	May 3, 1938	July 15, 1938	95	0	95	28
		July 15, 1939	94	19	75	31
May 18, 1937	----	July 15, 1937	34	4	30	18
	May 18, 1938	July 15, 1938	94	0	94	27
		July 15, 1939	88	6	82	24
June 3, 1937	----	July 15, 1937	45	20	25	13
	June 3, 1938	July 15, 1938	85	5	80	20
		July 15, 1939	90	20	70	24
June 18, 1937	----	July 15, 1937	40	5	35	15
	June 18, 1938	July 15, 1938	90	5	85	28
		July 15, 1939	89	21	68	23
July 3, 1937	----	July 15, 1937	50	17	33	14
	July 2, 1938	July 15, 1938	78	0	78	21
		July 15, 1939	94	12	82	31
July 15, 1937	----	July 15, 1937	21	5	16	37
	July 15, 1938	July 15, 1938	75	18	57	22
		July 15, 1939	89	18	71	32
Not defoliated	----	July 15, 1937	21	5	16	12
		July 15, 1938	79	10	69	29
		July 15, 1939	91	23	68	24
Aug. 3, 1937	----	Aug. 3, 1937	45	0	45	18
	Aug. 3, 1938	Aug. 3, 1938	88	13	75	21
		July 15, 1939	100	16	84	30
Not defoliated	----	Aug. 3, 1937	29	6	23	12
		Aug. 3, 1938	59	35	24	13
		July 15, 1939	100	0	100	34
Aug. 18, 1937	----	Aug. 18, 1937	20	5	15	16
	Aug. 18, 1938	Aug. 18, 1938	64	24	40	15
		July 15, 1939	95	20	75	29
Not defoliated	----	Aug. 18, 1937	5	0	5	11
		Aug. 18, 1938	44	21	23	13
		July 15, 1939	84	17	67	28
Sept. 3, 1937	----	Sept. 3, 1937	33	19	14	15
	Sept. 3, 1938	Sept. 3, 1938	33	33	0	0
		July 15, 1939	57	19	38	17
Not defoliated	----	Sept. 3, 1937	44	10	34	12
		Sept. 3, 1938	37	37	0	0
		July 15, 1939	74	26	48	18
Sept. 18, 1937	----	Sept. 18, 1937	34	29	5	17
	Sept. 19, 1938	Sept. 19, 1938	39	39	0	0
		July 15, 1939	71	14	57	22
Not defoliated	----	Sept. 18, 1937	15	10	5	7
		Sept. 19, 1938	50	15	35	15
		July 15, 1939	89	21	68	26
Oct. 2, 1937	----	Oct. 2, 1937	6	0	6	8
	Oct. 2, 1938	Oct. 3, 1938	42	38	4	8
	----	July 15, 1939	92	45	47	19
Not defoliated	----	Oct. 2, 1937	0	0	0	0
		Oct. 3, 1938	29	25	4	11
		July 15, 1939	96	38	58	24
Oct. 18, 1937	----	Oct. 18, 1937	21	21	0	0
	Oct. 18, 1938	Oct. 18, 1938	21	21	0	0
Not defoliated	----	Oct. 18, 1937	0	0	0	0
		Oct. 18, 1938	22	22	0	0

In recording inoculation results it seemed desirable to separate the deep lesions from the shallow ones in which the cortex only was involved. In the cortex type of lesion the fungus began to kill near the surface but did not kill as deep as the cambium; hence, the lesion healed over soon after active growth started. The deep lesions are probably much more important than the shallow ones, and therefore in table 2 the values for deep lesions rather than those for total infection should probably be given the main consideration in evaluating the results.

There was relatively little deep infection in 1937; but other inoculation experiments in that year also gave a low percentage of infection, indicating that that season was unfavorable for infection by *Xylaria*. The trees at that time were only in the third year from planting and were not crowding. Possibly the low infection of 1937 on defoliated and nondefoliated trees was due both to unfavorable conditions for the growth of *Xylaria* and to favorable conditions for growth of the host. A comparison of the percentages of deep lesions on the defoliated and the nondefoliated trees shows a tendency for infection to be more severe on the defoliated than on the nondefoliated trees.

The results were somewhat more striking the second year (1938), when sufficient disease developed to show considerable differences between treatments. Infection was heavy on all trees, but the highest percentage of deep infections was on trees defoliated in May. At the time of the May defoliation there were few new shoots and the leaves were only about half-size. After defoliation, new shoots rapidly pushed out and the trees soon had such a leafy appearance that one might have expected this early defoliation to have less effect than the later ones, when many more leaves were removed and renewals were slow and sparse.

The harmful effect of early defoliation on the tree is in accord with the observation of the writer that severe late-spring pruning after foliation is well on the way has a stunting effect. The deleterious effect of early defoliation is further shown by the fact that in 1939 trees defoliated early in the previous 2 years were decidedly stunted in comparison with trees on all the other plots.

DISCUSSION

In the experiment in which inoculations were made at monthly periods for more than 1 year, the time of greatest infection was July. Infection rose rapidly to its maximum and declined very abruptly in August and September (fig. 1). The results of the present experiment indicate that temperature should not be considered the main contributing factor to the shape of the curve of infection. It is possible that some host condition was responsible for greater susceptibility in midsummer than at any other time. Other experiments (*1*) indicate that *Xylaria mali* infects much more readily when the host is in a low state of vigor, and this suggests that susceptibility may be associated with a seasonal diminution in the root activity of the host. Resistance or susceptibility to attack by a disease organism is not known to have been reported heretofore as a measure of seasonal root activity.

The relation of host condition to infection by the perennial apple canker pathogen (*Neofabraea perennens* (Zeller and Childs) Kienholz) was noted by Shear and Cooley (*8*). This fungus attacked twigs during a very short period of the year, namely, in October, November, and December. This period of infectibility of apple twigs with this fungus corresponds in general to the period which Swarbrick (*10*) has shown to be the time of greatest starch accumulation and presumably of least activity of twigs as determined by carbohydrate changes. Results of the experiments reported in figure 1 indicate that there may be also a period of low vital activity of the roots and that maximum infectibility with a weak parasite such as *Xylaria mali* may indicate the time of the year when seasonal root activity of apple trees is lowest. Most of the reports of investigations on root development as determined by elongation show a period of greatly lessened activity during midsummer.

Kinman (*3*), working with drupaceous fruits in California, reported little or no root growth from June 1 to August 22. The physiological studies of Harris (*2*), Stevens (*9*), McDougall (*4*), and Rogers (*7*) on root activity of various tree species indicate that there is a period of relative inactivity in midsummer and a period of increased root activity in September and October. Reed and MacDougal (*6*) studied radial growth of orange trees in conjunction with shoot and root growth. Their data show a period of inactivity of the roots from early in June until about the middle of July. These periods of cessation of root activity of various plants correspond in general to the period of greatest susceptibility of apple roots to attack by *Xylaria mali*.

SUMMARY

Young apple trees in the nursery row were inoculated at monthly intervals from 1933 to 1935, inclusive, with the black root rot pathogen (*Xylaria mali*). In each year infection rose gradually to a maximum in July and declined greatly by September. It is suggested that the peak in infection may be related to a lessening of the metabolic activity of the roots in midsummer.

Maltreatment of the trees at monthly intervals, including cutting the trunk more than half through and bending the top over, ringing the trunk, root pruning, and summer pruning of branches, gave evidence that susceptibility to infection and the extent of the lesions are probably influenced by the seasonal activity of the trees. In general, the greatest effect of the maltreatments in increasing susceptibility was produced when the treatments were given in June and the inoculations made in July.

Artificial defoliation at periodic semimonthly periods was carried on for two summers and the residual effect was studied the third summer. Defoliation in general slightly increased susceptibility to the disease. Defoliations in the early part of the season resulted in more pronounced susceptibility to *Xylaria mali* and in a greater stunting of growth than defoliations in the latter part of the summer.

LITERATURE CITED

(1) Cooley, J. S.
1942. FACTORS AFFECTING DISTRIBUTION AND SEVERITY OF BLACK ROOT ROT OF APPLE TREES. Jour. Agr. Res. 65: 299–311.

(2) Harris, G. H.
1930. THE INFLUENCE OF TOP ON ROOT AS DETERMINED BY ROOT RESPIRATION OF YOUNG FRUIT TREES. Amer. Soc. Hort. Sci. Proc. (1929) 26: 329–334, illus.

(3) KINMAN, C. F.
1932. A PRELIMINARY REPORT ON ROOT GROWTH STUDIES WITH SOME ORCHARD TREES. Amer. Soc. Hort. Sci. Proc. 29: 220–224.

(4) MCDOUGALL, W. B.
1916. THE GROWTH OF FOREST TREE ROOTS. Amer. Jour. Bot. 3: 384–392.

(5) OLMSTEAD, L. B.
1928. SOIL TEMPERATURE RECORDS AT ARLINGTON FARM, VIRGINIA. Internatl. Cong. Soil Sci. Proc. and Papers (1927) 1: 539–546, illus.

(6) REED, H. S., and MACDOUGAL, D. T.
1937. PERIODICITY IN THE GROWTH OF THE ORANGE TREE. Growth 1: [371]–373, illus.

(7) ROGERS, W. S.
1939. ROOT STUDIES VIII. APPLE ROOT GROWTH IN RELATION TO ROOTSTOCK, SOIL, SEASONAL AND CLIMATIC FACTORS. Jour. Pomol. and Hort. Sci. 17: 99–130, illus.

(8) SHEAR, E. V., and COOLEY, J. S.
1933. RELATION OF GROWTH CYCLE AND NUTRITION TO PERENNIAL APPLE-CANKER INFECTION. (Abstract) Phytopathology 23: 33.

(9) STEVENS, C. L.
1931. ROOT GROWTH OF WHITE PINE (PINUS STROBUS L.). Yale Univ., School Forestry Bul. 32, 62 pp., illus.

(10) SWARBRICK, T.
1927. STUDIES IN THE PHYSIOLOGY OF FRUIT TREES. 1. THE SEASONAL STARCH CONTENT AND CAMBIAL ACTIVITY IN ONE- TO FIVE-YEAR-OLD APPLE BRANCHES. Jour. Pomol. and Hort. Sci. 6: 137–156, illus.

EFFECTIVENESS OF SELECTION ON PROGENY PERFORMANCE AS A SUPPLEMENT TO EARLIER CULLING IN LIVESTOCK[1]

By G. E. DICKERSON, *associate geneticist, Regional Swine Breeding Laboratory,* and L. N. HAZEL, *associate animal husbandman, Western Sheep Breeding Laboratory, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

The extensive literature on the use of the progeny test in selecting breeding animals deals almost exclusively with its accuracy, as compared with that obtained from the use of pedigree, individual performance, or averages of collateral relatives, as an indicator of transmitting ability. Although several investigators, particularly Wright (*15*)[3] and Lush (*3*, *4*, *5*), have emphasized difficulties in the practical use of the progeny test, its accuracy under properly controlled conditions is unquestioned. However, from the standpoint of genetic progress expected from selection in a given period of time, the usefulness of the progeny test is greatly influenced by factors other than its relative accuracy. The most important of these are the age at which progeny tests can be obtained and the rate of reproduction. The longer interval between generations that results from use of the progeny test in selection tends to offset the advantage of more accurate selection and may actually reduce the rate of improvement obtained.

The purpose of this study is to examine the effectiveness of selection based on the progeny test when it is used to supplement earlier selection. The criterion of effectiveness is the average genetic improvement expected yearly from early selection alone as compared with that expected when use is made of the progeny test. The examples have been chosen to include economic traits in farm livestock for which the basis of earlier culling is restricted to individual performance, pedigree, or average performance of collateral relatives.

ANNUAL IMPROVEMENT EXPECTED FROM SELECTION IN CLOSED POPULATIONS

The two factors that determine annual improvement from selection in any closed population are (1) the average genetic superiority of those animals selected to become parents over the group from which they were chosen (ΔP) and (2) the average age of parents when their offspring are born or the average interval between generations (T). These averages are weighted according to the proportion of offspring from parents of different sex and age groups. Since ΔP represents the average genetic gain in T years, the average annual gain is $\Delta G=\frac{\Delta P}{T}$.

---

[1] Received for publication April 28, 1943.

[2] The authors are indebted to Prof. W. G. Cochran of Iowa State College for suggestions on statistical procedure.

[3] Italic numbers in parentheses refer to Literature Cited, p. 475.

Journal of Agricultural Research,
Washington, D. C.

Vol. 69, No. 12
Dec. 15, 1944
Key No. A-218

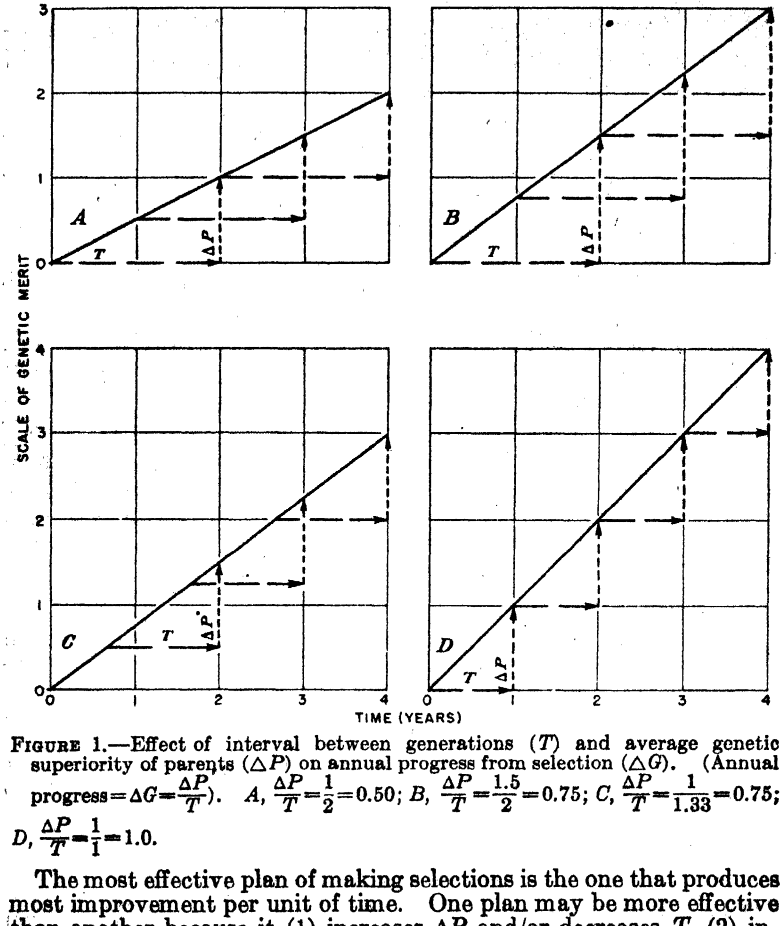


FIGURE 1.—Effect of interval between generations (T) and average genetic superiority of parents (ΔP) on annual progress from selection (ΔG). (Annual progress $= \Delta G = \frac{\Delta P}{T}$). A, $\frac{\Delta P}{T} = \frac{1}{2} = 0.50$; B, $\frac{\Delta P}{T} = \frac{1.5}{2} = 0.75$; C, $\frac{\Delta P}{T} = \frac{1}{1.33} = 0.75$; D, $\frac{\Delta P}{T} = \frac{1}{1} = 1.0$.

The most effective plan of making selections is the one that produces most improvement per unit of time. One plan may be more effective than another because it (1) increases ΔP and/or decreases T, (2) increases ΔP relatively more than T, or (3) decreases ΔP relatively less than T. The effect of changing ΔP and T is illustrated in figure 1.

The parents of the animals born in any one year differ in age and in the intensity of the selection applied to the different age groups and sexes. When the different parents within each age and sex group have equal opportunity to produce offspring, the average yearly genetic progress expected in two successive cullings of sires and of dams is[4]

$$\Delta G = \frac{\overbrace{N_1 \Delta S_1 + N_2(\Delta S_1 + \Delta S_2)}^{\Delta P \text{ for sires}} + \overbrace{M_1 \Delta D_1 + M_2(\Delta D_1 + \Delta D_2)}^{\Delta P \text{ for dams}}}{\underbrace{N_1 Y_1 + N_2 Y_2}_{T \text{ for sires}} + \underbrace{M_1 Z_1 + M_2 Z_2}_{T \text{ for dams}}} \qquad (1)$$

---

[4] This formula is rigorously proved algebraically from the fact that the average breeding value of an unselected group of offspring tends to be the same as that of the parents and may be extended to any number of successive cullings. The assumption is made that the average difference between offspring born in successive years (ΔG) is constant, as would be expected for polygenic traits in a closed population where a regular breeding plan was in use.

in which

ΔS_1=average genetic superiority of young sires retained in the first selection

N_1=proportion of the offspring that are from young sires

Y_1=average age of young sires when their offspring are born

ΔS_2=additional genetic superiority of sires obtained from the second culling of sires retained in the first selection

N_2=proportion of the offspring produced by sires retained in the second selection

Y_2=average age of sires saved in the second culling when their subsequent offspring are born

ΔD_1, M_1, Z_1, ΔD_2, M_2, and Z_2 have corresponding meanings for dams retained in the first and second cullings.

The general principles that govern progress from selection are the same for a whole breed as for a single closed herd. It is impractical to consider here the many forms that herd differences (genetic and environmental) may take, and they are ignored in the formulas for calculating ΔS_1, ΔS_2, etc. This procedure favors the progeny test, since the use of progeny averages helps to minimize errors in selection from random environmental variation within a herd but does not lessen those from environmental differences between herds unless a sire has progeny in more than one herd.

GENETIC SUPERIORITY FROM FIRST CULLING (ΔS_1 and ΔD_1)

In estimating the selection differential or average gain in apparent merit of a selected group as compared with the group from which it was chosen ($\bar{i}$), it is assumed that the basis of selection (I) is normally distributed and that all individuals below a given level are culled. The expected size of the differential or apparent gain in either sex depends on the proportion saved (p), as illustrated in figure 2. In

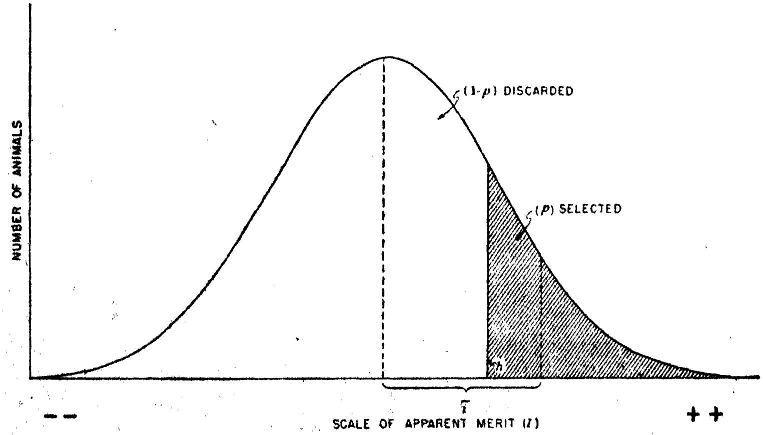


Figure 2.—A normal distribution showing how a population may be sharply divided at a point (h) into a selected (p) and a discarded ($1-p$) fraction. The average superiority in apparent merit of the selected fraction is ($\bar{i}$).

livestock breeding, p (and consequently $\bar{i}$) is largely determined by such factors as rate of reproduction, longevity, and age at puberty, peculiar to each type of livestock. Values of $\bar{i}$ in standard deviation units for different values of p are given for normally distributed populations of infinite size by Pearson (*10*) and may be calculated from Fisher and Yates (*1*) for smaller populations (from 2 to 50).

Since the selected group is chosen because of its superiority for some trait (X) or index (I_1) that is never perfectly correlated with transmitting ability (G), the average genetic superiority expected from the first selection is

$$\Delta S_1 \text{ or } \Delta D_1 = (\bar{i}_1) b_{GI_1} \sigma_{I_1} = (\bar{i}_1) r_{GI_1} \sigma_G. \tag{2}$$

Here $\bar{i}_1$ represents the selection differential in standard deviation units, b_{GI_1} the regression of transmitting ability on apparent merit, r_{GI_1} the corresponding correlation, and σ_G the standard deviation of transmitting abilities. It is convenient to calculate r_{GI_1} and σ_G in terms of the hereditary and environmental portions of the observed variance of the population; for example, when the first selection is made on some phenotypic trait (X),

$$r_{GX} = \sqrt{\frac{G}{E+G}} = \sqrt{G}, \text{ and } \sigma_G = \sigma_X \sqrt{G},$$

where G is the heritability or fraction of the observed variance caused by individual differences in transmitting ability $\left(\frac{\sigma_G^2}{\sigma_X^2}\right)$ and E is the remaining fraction attributed to environment, dominance, and epistasis or gene interaction $\left(\frac{\sigma_E^2}{\sigma_X^2}\right)$.

ADDITIONAL GENETIC SUPERIORITY FROM SECOND CULLING
(ΔS_2 AND ΔD_2)

All the culling possible may be done on the basis of the first information available, in which case ΔS_2, N_2, and Y_2 (or ΔD_2, M_2, and Z_2) become zero in formula (1). If the number of animals retained in the first culling permits a second culling after additional information (O), such as the progeny test, becomes available, the maximum additional genetic superiority from the second culling is

$$\Delta S_2 \text{ or } \Delta D_2 = (\bar{i}_2) R'_{G \cdot I_1 O} \sigma'_G. \tag{3}$$

Distinction is made between the multiple correlation ($R'_{G \cdot I_1 O}$) and standard deviation of transmitting abilities (σ'_G) among animals retained in the first culling, as compared with those in an unselected group ($R_{G.I_1O}$ and σ_G), because it is mathematically convenient to calculate the former in terms of the latter.[5]

The selection differential for the second culling ($\bar{i}_2$) can be calculated from table 20 of Fisher and Yates (*1*) since it is not affected materially by the slight skewness expected in the distribution of I_2.[6]

EFFECTIVENESS OF SELECTION FOR IMPORTANT TRAITS OF FARM ANIMALS

A regular plan of progeny testing may be effective in increasing rate of progress in one kind of animal but not in another even for similar traits, or for one kind of trait but not for another in the same population. The effectiveness depends largely on the age of parents when progeny-test information becomes available, but to some extent also on the rate of reproduction and the relative accuracy of information used for the first culling. Obviously these factors differ for particular kinds of animals and traits.

Several examples that illustrate the influence of these factors on the effectiveness of the progeny test have been chosen. These examples were selected because of their economic importance and because of the extensive breeding research that is being directed toward their improvement. They illustrate the effectiveness of the progeny test when used in conjunction with earlier selection based on pedigree, on individual performances, and on performance of collateral relatives, each of these plans being peculiarly fitted to making early selections for a different kind of trait.

TRAITS MEASURED IN BOTH SEXES BEFORE BREEDING AGE

Many important traits, such as growth rate, economy of feed utilization, market conformation, fleece weight, and fleece length, can be

---

[5] The standard deviation of transmitting abilities among the group saved in the first culling is

$$\sigma'_G=\sigma_G\sqrt{1-r^2_{GI}\ (1-\sigma^2_s)}$$

where σ^2_s is the fraction of the original variance of I_1 that remains in the selected group. Values of σ^2_s (hereafter designated as σ^2_{ss} for sires and σ^2_{sd} for dams) may be calculated for large populations from the formula $\sigma^2_s=1-\bar{i}_1(\bar{i}_1-h)$, suggested by Professor Cochran, where h is the plus or minus deviation from the mean of the unselected population at the point of truncation of the normal curve (fig. 2).

The multiple correlation of G with O and I_1 among animals retained in the first culling is

$$R'_{G\cdot OI_1}=\sqrt{\frac{r^{2'}_{GI_1}+r^{2'}_{GO}-2r^{2'}_{GI_1}r^{2'}_{GO}}{1-r^{2'}_{GI_1}r^{2'}_{GO}}}$$

The correlation between G and I_1 among those selected in the first culling is

$$r'_{GI_1}=r_{GI_1}\sqrt{\frac{\sigma^2_s}{1-r^2_{GI_1}(1-\sigma^2_s)}}$$

whereas that between G and O is

$$r'_{G_sO_s}=r_{G_sO_s}\sqrt{\frac{1-r^2_{GI_1}(1-\sigma^2_{ss})}{1-r^2_{GI_1}r^2_{G_sO_s}(1-\sigma^2_{ss})-r^2_{GI_1}r^2_{G_dO_s}(1-\sigma^2_{sd})}}$$

for sires, and

$$r'_{G_dO_d}=r_{G_dO_d}\sqrt{\frac{1-r^2_{GI_1}(1-\sigma^2_{sd})}{1-r^2_{GI_1}r^2_{G_sO_d}(1-\sigma^2_{ss})-r^2_{GI_1}r^2_{G_dO_d}(1-\sigma^2_{sd})}}$$

for dams.

[6] The exact selection differential expected in the second culling ($\bar{i}_2$) of a population of infinite size was calculated by a method, suggested by Professor Cochran, for varying proportions retained in the first (p_1) and second (p_2) culling and for different degrees of correlation between I_1 and I_2 in the unselected population. Even when $r_{I_1I_2}$ is as large as 0.8, the exact value of $\bar{i}_2$ expected does not differ appreciably, because of skewness, from that expected for a normal distribution unless p_2 is much larger or smaller than 0.5. For example, when $p_1=0.2$, $p_2=0.1$, and $r_{I_1I_2}=0.8$, the exact expectancy for $\bar{i}_2$ is only 3 percent higher than for a normal distribution. In the examples that follow, p_2 is never larger than 0.5 nor smaller than 0.1 and $r_{I_1I_2}$ does not exceed about 0.8.

measured on both males and females before they reach breeding age. The annual progress expected from selection based on individual performance alone in swine and sheep as compared with that expected from the supplementary use of the progeny test is indicated in the examples that follow.

When first selections are based on individual performance (X) alone, the genetic superiority expected from the first selection is

$$\Delta S_1 \text{ or } \Delta D_1 = (\bar{i}_1) G\sigma_X. \qquad (4)$$

When second selections are based on the optimum combination of individual performance and progeny test (formula 3), the additional genetic superiority expected is

$$\Delta S_2 = (\bar{i}_2) G\sigma_X \sqrt{\sigma_{ss}^2 + \frac{nd(1-G)^2}{4(A+nB)+ndG(1-G)-nG^2(1-\sigma_{sd}^2)}} \qquad (5)$$

for sires, and

$$\Delta D_2 = (\bar{i}_2) G\sigma_X \sqrt{\sigma_{sd}^2 + \frac{n(1-G)^2}{4(A+nB)+nG(1-G)-nG^2(1-\sigma_{ss}^2)}} \qquad (6)$$

for dams, each of which produces one litter of n progeny. The symbols and their interpretations in terms of the hereditary and environmental fractions of the variance are as follows:

L=fraction of total variance due to differences in environment and in gene interaction that are alike for members of the same litter
E=fraction that behaves as random environmental variation between litter mates
A=fraction due to differences between litter mates=$E+G/2$
B=fraction due to differences between paternal half-sibs, less $A=L+G/4$
C=fraction due to differences between nonsibs, less $A+B=G/4$, so that $G+L+E=A+B+C=1$

In addition,
n=number of offspring per litter, and
d=number of litters per sire.

Weight at 180 days may be used as an example for swine, in which $G=0.30$, $L=0.20$, $E=0.50$, and $\sigma_x=32$ pounds, in accord with values found by Whatley (*14*) and Hazel (*2*). We shall consider a 20-sow herd in which breeding stock are saved from the spring farrow only and $n=5$. The age when offspring are born is 1 year for young boars and gilts and 2 years for the tested boars and sows. The selection differentials for formulas (4), (5), and (6) are calculated from table 20 of Fisher and Yates (*1*). For example, if 3 young sires are saved annually, $p=3/50$, so that, for sires, $(\bar{i}_1)=1.91$. When the best 1 of the 3 is kept after testing, $p=1/3$ and $(\bar{i}_2)=0.85$. The curves for annual progress in figure 3 were constructed from formula 1. Solid lines show progress expected from the use of 2, 3, and 4 young sires and 1 boar tested the year before, when no tested sows are used ($M_2=0$), but the proportion of the litters by the tested boar (N_2) varies from 0 to 0.8. Broken lines show progress expected as the proportion of litters farrowed by 2-year-old sows (M_2) varies from 0 to 0.5, when all litters are sired by 2 young boars ($N_2=0$).

Figure 3, *A*, (solid lines) shows that ΔG is maximum when 2 young sires are used each year on all sows. The use of 3 or 4 young sires decreases progress, as does increasing the proportion of offspring by tested sires. The use of 1 young sire instead of 2 would not increase ΔG since all offspring would then be paternal half-sibs, and the reduced genetic variability and heritability would more than cancel the slightly larger selection differential. Even a plan of testing the optimum number of 6 to 8 sires in an auxiliary herd of 20 sows and using the 2 best ones in the closed herd does not increase ΔG, as shown at the extreme right of figure 3, *A*. This indicates that the progeny test for sires is not effective for this or similar traits in swine, regardless of herd size.

Figure 3, *A*, (broken lines), also shows that ΔG is about the same when the optimum proportion of the litters (10 to 20 percent) is from

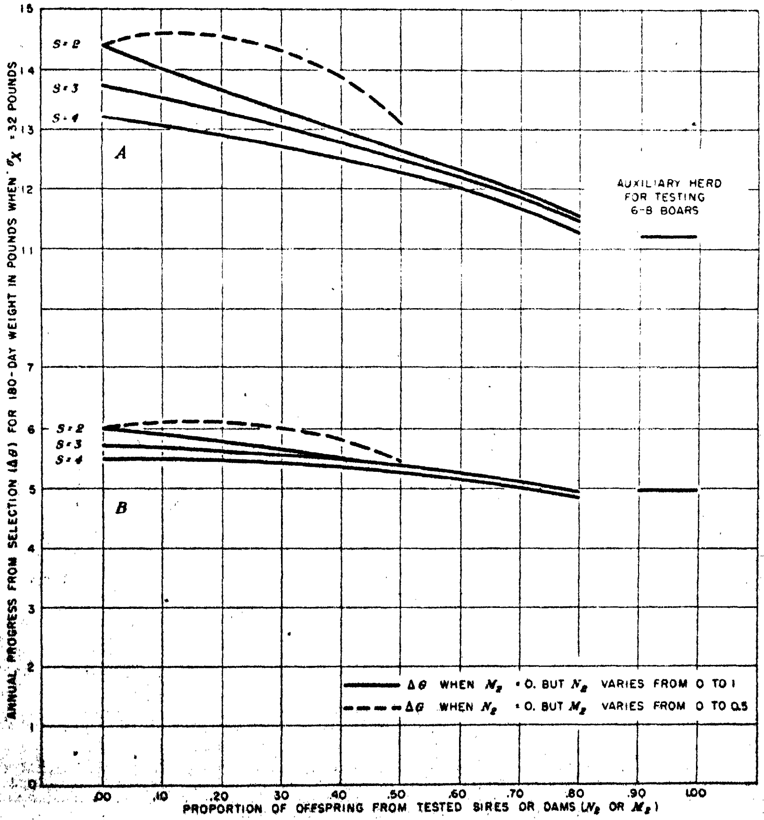


FIGURE 3.—Effect of progeny testing on genetic progress from selection for 180-day weight in a closed 20-sow herd of swine, when early culling is based on individual weights. *A*, When $G=0.30$, $L=0.20$; *B*, when $G=0.125$, $L=0.25$. s=number of sires tested each year.

tested sows as when only gilts are used. As M_2 is increased, the first selection of gilts becomes more effective, the second selection of sows becomes less effective, and the average age of dams increases. Up to $M_2=0.2$ the opposing influences nearly cancel, but as M_2 is increased further, progress declines.

It is apparent from formulas (5) and (6) that progeny testing is more likely to increase progress for traits of lower heritability (G smaller, E larger) that are unaffected by litter environment ($L=0$, $B=G/4$). As a population becomes more unform genetically owing to inbreeding (f), heritability declines (that is, $G\cong\frac{G_o(1-f)}{1-G_of}$). For example, as f rises from 0 to 0.67, the heritability (G) of growth rate in swine would change roughly from 0.30 to 0.125, L from 0.20 to 0.25, and E from 0.50 to 0.625. Figure 3, *B*, shows that progeny testing of boars or sows does not increase ΔG even at this lower level of heritability, although the reduction in ΔG from the use of a tested boar is less marked. G and L appear to be a little smaller for conformation score at market weight, as shown by Stonaker and Lush (*12*), than for growth rate of swine, but not enough so to make use of progeny-tested sires advantageous.

The influence that rate of reproduction has on the effectiveness of the progeny test may be illustrated by comparing selection for body weight or fleece length in yearling sheep with that for growth rate in swine. The influence of the time required to obtain progeny tests of sires is shown by comparing selection for yearling traits with that for weanling traits in sheep. Table 1 shows the age distribution, fertility, and average age of dams expected in a flock of 100 ewes if all voluntary culling of females were done before breeding age. In the first selection the best 44 percent of the 50 ewe lambs or yearling ewes and the best 2, 3, or 4 of the 50 ram lambs or yearling rams are chosen. Formulas (4) and (5) may be used in calculating ΔD_1, ΔS_1, and ΔS_2 ($\Delta D_2=0$, $n=1$, and $A+nB=1-G/4$).

TABLE 1.—*Age distribution, fertility, and average age of dams at lambing time in a flock of 100 ewes, when all voluntary culling is done before breeding age* [1]

Age of females (years) (1)	Ewes of each age per 100 breeding ewes (2)	Lambs weaned per ewe (3)	Fraction of all lambs weaned = $\frac{(2)\times(3)}{100}$ (4)	T for ewes [(1)×(4)] (5)
	Number	*Number*		*Years*
0	22.0			
1	21.4			
2	20.9	0.62	0.129	0.259
3	19.7	.93	.184	.552
4	18.3	1.22	.222	.893
5	16.4	1.17	.192	.960
6	13.8	1.12	.153	.924
7	10.9	1.10	.120	.842
Total	143.4		1.000	4.430

[1] These data were taken from a report by Terrill (*13*) on the Rambouillet flock of the United States Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho. They have been adjusted for voluntary culling after breeding age and for a 100-percent lamb crop at weanling age. The figures for ewe lambs and yearling ewes represent those necessary for replacements.

Figure 4, *A*, shows yearly progress for weanling (solid lines) and for yearling (broken lines) traits when heritability is 0.30 and rams are used first as yearlings. Use of the best ram tested the year before on the optimum proportion (0.6 to 0.7) of the ewes increases progress by about 4 percent for weanling traits but reduces progress for yearling traits, as compared with the use of only the 2 best yearling rams each year. The difference occurs solely because selection of rams on progeny performance can be made a year earlier for weanling than for yearling traits. As shown at the extreme right of the figure a still greater increase in progress (6 percent) could be obtained for weanling traits by testing the optimum number (7) of yearling rams each year in an auxiliary flock of 100 ewes and using the 2 best ones

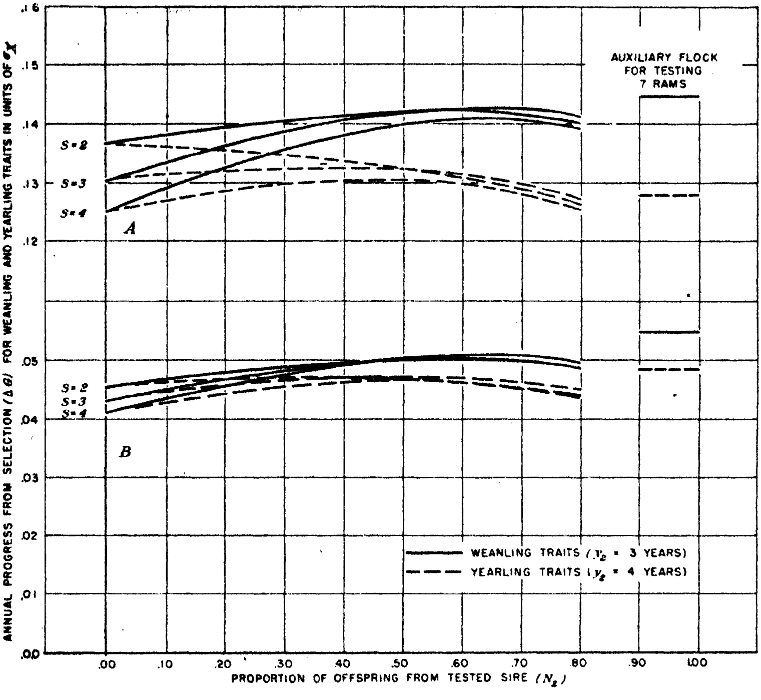


FIGURE 4.—Influence of progeny testing of rams on genetic progress from selection for weanling and yearling traits in a closed 100-ewe flock of sheep, when early culling is based on individual performance. *A*, When $G=0.30$, $L=0$; *B*, when $G=0.10$, $L=0$. s=number of sires tested each year.

in the main flock the following year as 2-year-olds. This plan is not effective for yearling traits, however.

When heritability is only 0.10 (fig. 4, *B*) use of the best progeny-tested ram is expected to increase progress about 11 percent for weanling traits and 3 percent for yearling traits. By using the auxiliary flock for testing rams, the increase in progress would be raised to 20 percent for weanling traits and 5 percent for yearling traits.

Progress for weanling traits could be increased still further by testing each of the best ram lambs on a small number of ewes and then using the best tested rams as yearlings instead of as 2-year-olds.

For example, suppose the 4 best ram lambs were tested on 40 ewes and the remaining 60 ewes were mated to the best yearling ram tested the year before. Under this plan, yearly progress expected would be 20 and 28 percent greater, for heritabilities of 0.3 and 0.1, respectively, than if the 2 best untested yearling rams were used on the entire flock, whereas the advantage of testing yearling rams and using the best tested 2-year-old is only 4 and 11 percent, respectively. The increase in progress from testing rams in the auxiliary flock of 100 ewes is also greater if the tested rams can be used in the main flock as yearlings instead of as 2-year-olds (22 and 37 percent, respectively, for heritabilities of 0.3 and 0.1, instead of 6 and 20 percent). These maximum estimates of the gain in rate of improvement from using progeny-tested rams are far below the 500 percent gain claimed by McMahon (*9*) for heritability at 0.10. The discrepancy appears to be due largely to McMahon's assumptions that (1) the average of 7 progeny is perfectly correlated with the sire's genotype and (2) the interval between generations would not be lengthened by using tested rams. Actually, the correlation of a ram's genotype with the average of 7 progeny would be little larger ($r_{GO}=0.39$) than with the ram's own phenotype ($r_{GX}=\sqrt{0.10}=0.32$), in an unselected population and for heritability at 0.10. For weanling traits, the generation interval could actually be shortened a little, compared with the use of untested yearling rams, by testing ram lambs on part of the flock and using tested rams as yearlings on the rest of the ewes. However, progeny tests for yearling traits on rams used as lambs or yearlings would be obtained only in time for use of selected tested rams as 2- or 3-year-olds, and use of tested rams would lengthen the generation interval by 6 months or 1 year compared with the use of untested yearling rams.

TRAITS MEASURED ONLY AFTER SLAUGHTER

Information on collateral relatives may not be of sufficient importance for traits measured in both sexes before breeding age to be considered in making selections. For traits measurable only in the carcass, there may be no other basis for making early selections. In a closed herd of swine, for example, several pigs from each litter may be slaughtered at market weight, the information being used in selecting collateral relatives and for progeny-testing the previous group of young sires.

The genetic superiority for a carcass trait (X) of boars or gilts selected in the first culling based on the average of n litter mates is

$$\Delta S_1 \text{ or } \Delta D_1=(\bar{i}_1)\frac{G}{2}\sigma_X\sqrt{\frac{n}{A+n(B+C)}}. \tag{7}$$

The additional genetic superiority from the second culling based on the optimum combination of the average of nd progeny and of n litter mates is

$$\Delta S_2=(\bar{i}_2)\frac{G}{2}\sigma_X\cdot$$

$$\sqrt{\frac{n}{A+n(B+C)}\left\{\sigma_{ss}^2+\frac{d(A+nB)^2}{(A+nB+ndC)[A+n(B+C)]-n^2C^2(d+1-\sigma_{ss}^2)}\right\}} \tag{8}$$

for sires, and

$$\Delta D_2 = \overline{(i}_2)\frac{G}{2}\sigma x\sqrt{\frac{n}{A+n(B+C)}\left\{\sigma_{sd}^2+\frac{(A+nB)^2}{[A+n(B+C)]^2-nC^2(2-\sigma_{ss}^2)}\right\}} \tag{9}$$

for dams having only one litter. The symbols used are those defined for formulas (4) to (6).

The curves in figure 5 are for a closed 20-sow herd in which carcass traits are observed on 2 pigs from each litter and when the s young boars and the gilts used for breeding each year are chosen from 40

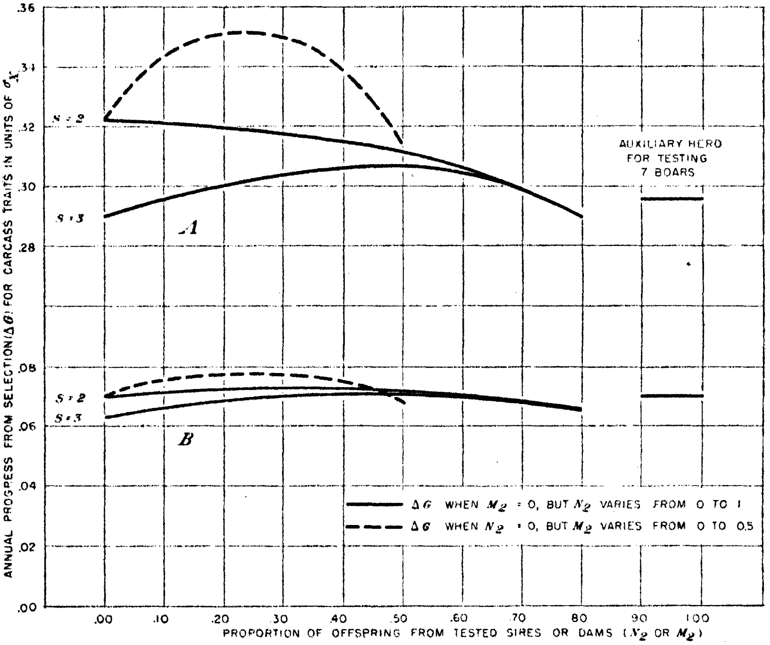


FIGURE 5.—Influence of progeny testing on genetic progress from selection for carcass traits in a closed 20-sow herd of swine, when the first culling is based on averages for 2 litter mates. *A*, When $G=0.50$, $L=0$; *B*, when $G=0.10$, $L=0$. s = number of sires tested per year.

gilts in 20 litters and 10 boars in 10 litters. Use of the best sire tested the preceding year reduces progress expected when heritability is 0.50 (fig. 5, *A*), although progress is increased about 10 percent if optimum proportion of the litters (about 0.2) is from the best sows tested the year before. When heritability is as low as 0.10 (fig. 5, *B*), the progeny test is slightly effective. As shown at the extreme right of figure 5, progress is not increased by testing the optimum number (7) of boars in an auxiliary herd of 20 sows and using the best 2 in the main herd the next year.

Thus it seems unlikely that progeny testing can increase progress for carcass traits in swine appreciably if earlier culling of breeding animals can be based on the average performance of several litter mates. However, progeny tests are much more likely to be helpful in selecting for such traits in beef cattle or sheep, where the reproductive rate is lower and earlier culling must be based on the performance of half-sibs rather than full sibs.

REPEATABLE TRAITS EXPRESSED ONLY IN THE FEMALE

Progeny testing of sires might be presumed to have special usefulness in selecting for such traits as butterfat production in dairy cattle or prolificacy in swine, since early culling must be based largely on pedigree. Because progeny tests have received so much attention both in the literature and in the practice of dairy-cattle improvement, butterfat production has been used in the example that follows.

The genetic superiority for butterfat production (X) expected for bull or heifer calves selected in a first culling based on an average of k'' records for each of the dams is

$$\Delta S_1 \text{ or } \Delta D_1 = (\bar{i}_1)\frac{G}{2}\sigma_X\sqrt{\frac{k''}{E_r+k''[E_p+G]}}, \tag{10}$$

where the intraherd variance in butterfat production (σ_X^2) is subdivided into the following fractions:

G = heritability or fraction due to differences in transmitting ability

E_p = fraction due to permanent differences in environment, to deviations from transmitting ability due to dominance and epistasis, and

E_r = fraction due to random variation in environment between different records of the same cow, after adjustment for age.

The additional genetic superiority expected for sires selected in a second culling based on the best combination (I_2) of the average production for d daughters with k records each and the average of k'' records of each sire's dam is (from formula 3)

$$\Delta S_2 = \frac{(\bar{i}_2)G\sigma_X}{2\sqrt{E_r+k''(E_p+G)}}\cdot \sqrt{k''\sigma_{ss}^2+\frac{kd[4(E_r+k''E_p)+3k''G]^2}{4[E_r+k''(E_p+G)][4(E_r+kE_p)+kG(d+3)]-kk''G^2(d+1-\sigma^2_{sd})}}. \tag{11}$$

The additional genetic superiority expected for dams selected in a second culling based on the best combination of each cow's own average for k' records and her dam's average for k'' records is

$$\Delta D_2 = \frac{(\bar{i}_2)G\sigma_X}{2\sqrt{E_r+k''(E_p+G)}}\cdot \sqrt{k''\sigma_{sd}^2+\frac{k'[4(E_r+k''E_p)+3k''G]^2}{4[E_r+k''(E_p+G)][E_r+k'(E_p+G)]-k'k''G^2}}. \tag{12}$$

The results to be expected from progeny testing of dairy sires are shown in figure 6 for a closed herd of 120 cows. Heritability (G) is 0.25 in figure 6, *A*, and 0.10 in *B*, but repeatability ($G+E_p$) is 0.35 in both. These are roughly the upper and lower limits of heritability indicated in such studies as those of Lush, Norton, and Arnold (*7*) and Lush and Straus (*8*). In this example, one-fourth of the cows are replaced each year; only calves from three-fourths of the cows that have completed one or more records are considered in selecting breeding animals; and 90 percent of the cows raise calves each year. Three-fourths of the heifer calves (30/40) are saved in the first culling and kept for two lactations, after which one-half (15/30) are retained in the second culling for an average of 4 more lactations. Thus the average age of dams when the calves from which breeding stock are chosen (second record and later) are born is about $4\frac{2}{3}$ years; that is, $\frac{3\text{ years}}{3}+\frac{2}{3}$ (5.5 years). The average number of records per dam is $k''=2\frac{2}{3}$. Also, young sires selected for progeny testing and used for 1 year when from 15 to 27 months of age are about $2\frac{1}{2}$ years old when their calves are born (that is, $Y=2\frac{1}{2}$ years). When the 1 best sire tested over each 2-year period (on the basis of d daughters with $k=1$ record each) is used again on part of the herd for a 2-year period, his average age when his second group of calves is born will be about 8 years (that is, $Y_2=8$ years). Under these conditions, the annual progress expected is

$$\Delta G_t=\frac{N_1(\Delta S_1)+N_2(\Delta S_1+\Delta S_2)+\frac{1}{3}(\Delta D_1)+\frac{2}{3}(\Delta D_1+\Delta D_2)}{2.5N_1+8N_2+4\frac{2}{3}}.$$

Figure 6 shows that the progeny test is not effective under the conditions stated, ΔG_t decreasing as N_2 increases.

The total annual progress (ΔG_t) is subdivided in figure 6 into that from the two successive cullings among females (ΔG_d) and that from the two cullings among males (ΔG_s). As Seath (*11*) has shown, much of the culling among females is for disease, breeding failure, and similar causes, so that ΔG_d for butterfat production actually may be considerably less than indicated in figure 6. This would make the curves for ΔG_t more nearly like those for ΔG_s, which show the progeny test in a more favorable light. If no progress is made from selection of dams ($\Delta G_d=0$), progress from sire selection (ΔG_s) is optimum when one-third to one-half of the cows are bred to the best 1 of 4 sires tested during the 2 preceding years, and ΔG_s is increased about twice as much (10 percent as compared with 5 percent) by progeny testing when heritability is 0.10 as when heritability is 0.25.

Use of progeny-tested dairy sires would be a little more likely to increase the rate of improvement if, instead of the average production of the daughters alone being used, that of the dams to which each sire was mated were also considered properly. This procedure, according to Lush (*6*), would make progeny tests in a population of many herds about 1.12 to 1.20 times as accurate as the use of the average production of the daughters alone. The exact amount depends largely on the correlation between the average production of the sire's daughters and that of their dams, and would, therefore, be less for comparisons between sires tested in the same herd and during the same years. In the above example (when selection of dams is presumed ineffective), increasing the accuracy of progeny tests 1.10 times would change the percentage increase in yearly improvement resulting from optimum use of tested sires only, from 10 to 13 if heritability is 0.10 and from 5 to 8 if heritability is 0.25.

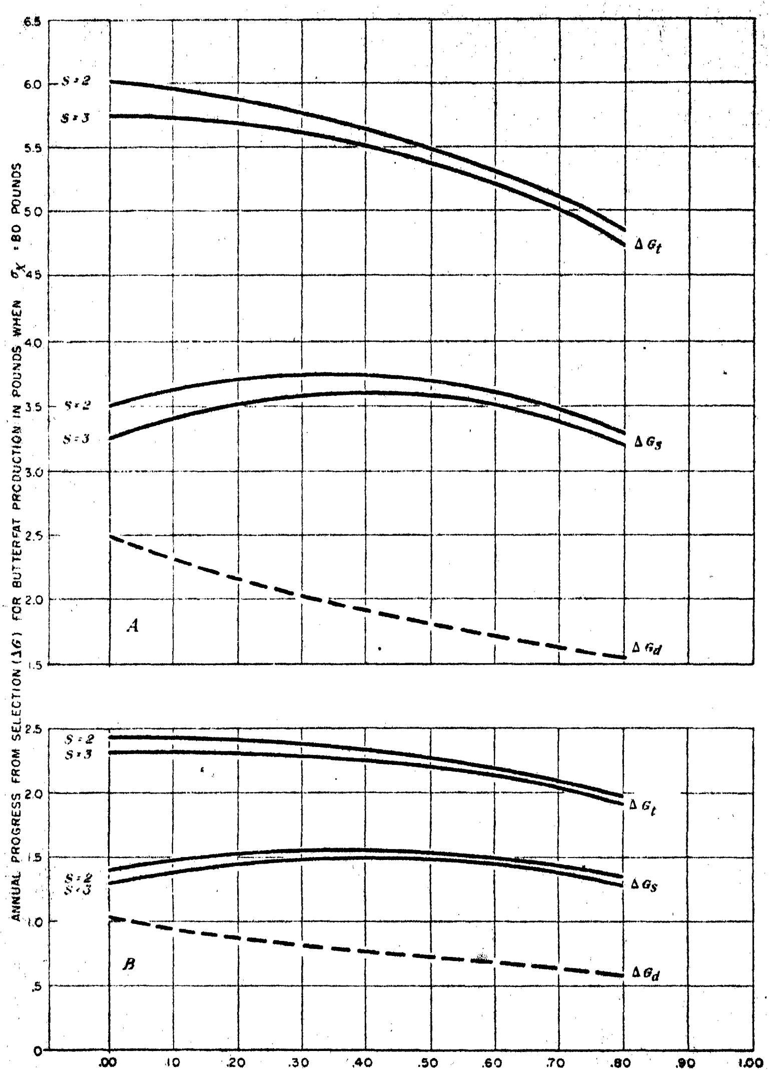


FIGURE 6.—Effect of using progeny-tested sires on genetic progress from selection for butterfat production in a closed 120-cow herd, when early culling i based on the dam's average production alone. *A*, When $G=0.25$, $E_p=0.10$; *B*, when $G=0.10$, $E_p=0.25$. s = number of sires tested each year.

DISCUSSION

The foregoing examples indicate that the possibilities of increasing progress by a regular plan for use of progeny-tested sires are limited to certain kinds of livestock and to certain traits. The reasons for this limitation may be illustrated by comparing the effectiveness of progeny testing in the different examples. First, the less the interval between generations is increased by progeny testing the more likely it is that progeny testing will increase progress. This is shown by contrasting the results of selecting for weanling and yearling traits in sheep (fig. 4). The only difference in these examples is that 1 year is required to obtain progeny-test information on weanling traits, whereas 2 years are required for yearling traits. Second, when the rate of reproduction is low, progeny testing of sires is more likely to increase progress. The resulting increase in genetic superiority of parents (ΔP) tends to be larger, relative to that in the age of parents (T), when there is less opportunity for early culling, particularly among females. This is the reason that progeny testing affects progress more favorably for yearling traits in sheep (fig. 4) than for growth rate in swine (fig. 3), and when little voluntary culling of females is possible for butterfat production in dairy cattle (ΔG_4 in fig. 6). Third, if the basis for making first selections is relatively inaccurate, the progeny test is more likely to be effective, because there is more environmental variation to be discounted by the progeny test and more of the genetic variation remains among animals tested. This is illustrated by the contrast in the effectiveness of the progeny test for high and low heritability (figs. 3, 4, 5, and 6).

Thus a combination of circumstances, largely beyond the breeder's control, operates to make the use of a regular plan of progeny testing a wise or unwise procedure. In many cases when the progeny test is most easily applied it may actually reduce genetic progress (fig. 3). Even when the circumstances indicate the use of progeny-tested sires, there is danger that its full effectiveness will not be realized in practice because of unwise judgment. Too many or too few young sires may be tested on too many or too few females, so that the optimum use of young and tested sires is not attained (fig. 4). Although little attention has been given to this point, it becomes important once a breeder decides to use a regular plan of progeny testing.

The progeny test is not likely to be more effective in increasing genetic progress in actual practice than under the conditions assumed in the foregoing examples. Where assumptions had to be made, these generally favored the progeny test. For example, the average of a sire's offspring was assumed to be unbiased because of special treatment or selection among the offspring before the trait (X) was measured. Although the sharp truncation assumed here is not likely to exist in actual practice, particularly for any single trait, this seemingly overestimates the selection differential for all age groups in both sexes. This favors the older animals, since more culling for age, sterility, disease, and other factors and more deaths would occur among them.

It was necessary to assume that the genetic gain from the first and the second selections (ΔS_1 and ΔS_2 or ΔD_1 and ΔD_2) was constant from year to year. Of course, this would not be exactly true, particularly for small herds. Nevertheless, the estimates of ΔG in the foregoing examples represent the average expectancy for any of the plans for regular use of progeny-tested sires. The effectiveness of progeny testing would be somewhat greater than indicated in these examples if a tested sire were used only when one of exceptional merit was found, or if the proportion of the herd mated to the tested sire were varied according to his apparent superiority. Although there are some notable cases in which the progeny test has identified animals of exceptional transmitting ability, these cases are rare in breed histories and offer uncertain possibilities to the individual breeder who can test only a limited number of sires. Breeders who regularly use the best young sires extensively would be better able to recognize and make use of the occasional outstanding sire, if still available, than breeders who regularly use a tested sire on most of the herd and use young sires sparingly.

For simplicity, selection for one trait at a time was considered in the examples. Methods have been developed by Hazel (*2*) for expressing net merit as a linear function of several traits, including the performance of relatives. The relative effectiveness of the progeny test in selecting for an index based on several traits would be much the same as that for a single trait. In fact, the formulas developed herein for individual traits could be applied directly to more complicated cases by considering the index as a single trait.

The effect of progeny testing on the genetic progress in an entire breed is much the same as in a closed herd. A breed is just a much larger closed herd, in which another source of variation, herd differences, must be considered and in which inbreeding may be a negligible factor. Thus the same general conclusions for selection within closed herds apply for the much larger closed population of an entire breed. The regular use of progeny tests does not increase, and may decrease, the rate of progress unless the progeny-test information can be obtained early, the reproductive rate is low, and there is little or no basis for earlier culling.

These conclusions do not conflict in any way with the fact that unbiased progeny-test information always increases the accuracy of selection for traits that are influenced much by dominance, epistasis, or environmental variations. They simply mean that in the time required to carry out the progeny test the genetic progress from selection based on pedigree, individual merit, or family averages may be more than that obtained from selection on the progeny test. For the improvement of most traits, in most kinds of livestock, these conclusions point unmistakably toward the fuller use of pedigree, individual merit, and family averages for early culling in order to keep the interval between generations short and progress maximum.

The technique of artificial insemination may increase the advantage of using selected progeny-tested sires if the population is sufficiently large and if the reproductive rate of males is increased markedly thereby, as in sheep and cattle. If fewer sires are needed, more progress is expected from the more intense selection of young sires. However, mating each young sire to larger numbers of females also is likely to increase the accuracy of selecting between tested sires more than enough to offset the increased intensity selection of young sires, particularly for traits low in heritability.

In the literature, progeny testing does not always refer to use of progeny-tested individuals. The progeny-test breeding so successfully practiced on laboratory animals, poultry, and plants is often based on selection between and within the progenies themselves rather than between parents on the basis of the progeny test, and consequently does not increase the interval between generations. Actually, this is selection based on a combination of individual performance and family average. It differs from the methods indicated for farm livestock only in the greater emphasis on selection between families or progenies which a higher reproductive rate permits.

SUMMARY AND CONCLUSIONS

Annual improvement from selection in a closed herd or breed is the ratio of the average genetic superiority of parents (compared with the unselected group from which they were chosen) to the average age of parents when offspring are born.

Examples of progress expected from selection based on pedigree, individual performance, or averages for collateral relatives, with and without the supplementary use of the progeny test, have been given for representative economic traits of farm animals.

A regular plan of progeny testing is unlikely to increase, and may reduce, progress unless (1) the progeny-test information becomes available early in the tested animal's lifetime, (2) the reproductive rate is low, and (3) the basis for making early selections is relatively inaccurate. These factors are largely beyond the breeder's control, being relatively unchangeable for a particular kind of animal and trait.

Opportunity for improvement from selection is nearly maximum for most traits when (1) culling is based on individual performance, family average, and pedigree and (2) the interval between generations is kept short. Possible exceptions are weanling traits in sheep and carcass traits in sheep and beef cattle.

LITERATURE CITED

(1) Fisher, R. A., and Yates, F.
1938. STATISTICAL TABLES FOR BIOLOGICAL, AGRICULTURAL AND MEDICAL RESEARCH. 90 pp. London and Edinburgh.

(2) Hazel, L. N.
1943. THE GENETIC BASIS FOR CONSTRUCTING SELECTION INDEXES. Genetics 28: 476–490, illus.

(3) Lush, J. L.
1933. THE BULL INDEX PROBLEM IN THE LIGHT OF MODERN GENETICS. Jour. Dairy Sci. 16: 501–522, illus.

(4) ———
1935. PROGENY TEST AND INDIVIDUAL PERFORMANCE AS INDICATORS OF AN ANIMAL'S BREEDING VALUE. Jour. Dairy Sci. 18: 1–19, illus.

(5) ———
1936. GENETIC ASPECTS OF THE DANISH SYSTEM OF PROGENY-TESTING SWINE. Iowa Agr. Expt. Sta. Res. Bul. 204, 196 pp., illus.

(6) ———
1944. THE OPTIMUM EMPHASIS ON DAMS' RECORDS WHEN PROVING DAIRY SIRES. Jour. Dairy Sci. (In press.)

(7) ——— Norton, H. W., III, and Arnold, F.
1941. EFFECTS WHICH SELECTION OF DAMS MAY HAVE ON SIRE INDEXES. Jour. Dairy Sci. 24: 695–721, illus.

(8) ——— and Straus, F. S.
1942. THE HERITABILITY OF BUTTERFAT PRODUCTION IN DAIRY CATTLE. Jour. Dairy Sci. 25: 975–982.

(9) McMahon, P. R.
1940. INCREASED PROFITS FROM SHEEP THROUGH PROGENY TESTING AND CULLING FOR PRODUCTION. Massey Agr. Col., Palmerston, New Zeal. Sheep Farmers Proc. 9: 37–49, illus.

(10) Pearson, K., ed.
[1931]. TABLES FOR STATISTICIANS AND BIOMETRICIANS. Pt. 2, ed. 1, 262 pp., illus. Cambridge.

(11) Seath, D. M.
1940. THE INTENSITY AND KIND OF SELECTION ACTUALLY PRACTICED IN DAIRY HERDS. Jour. Dairy Sci. 23: 931–951, illus.

(12) STONAKER, H. H., and LUSH, J. L.
1942. HERITABILITY OF CONFORMATION IN POLAND-CHINA SWINE AS EVALUATED BY SCORING. Jour. Anim. Sci. 1: [99]-105.
(13) TERRILL, C. E.
1939. SELECTION OF RANGE RAMBOUILLET EWES. Amer. Soc. Anim. Prod. Proc. (1939) 32: 333-340.
(14) WHATLEY, J. A., Jr.
1942. INFLUENCE OF HEREDITY AND OTHER FACTORS ON 180-DAY WEIGHT IN POLAND CHINA SWINE. Jour. Agr. Res. 65: 249-264, illus.
(15) WRIGHT, S.
1932. ON THE EVALUATION OF DAIRY SIRES. Amer. Soc. Anim. Prod. Proc. (1931) 24: 71-78.

U. S. GOVERNMENT PRINTING OFFICE: 1944

INDEX

Page
Abbe, Ernst, C., Randolph, L. F., and Einset, John: Comparison of Shoot Apex and Leaf Development and Structure in Diploid and Tetraploid Maize 47–76
Acorns, chemical analyses 37, 41–42, 44–45
Actinomyces scabies—
  common scab fungus, variation and physiologic specialization. L. A. Schaal 169–186
  cultural variation 172–178
  cultures—
    designation 170
    effects of nutrients, experiments 174–175
    pathogenicity tests 178–182, 185
    sectoring frequency 172–173
  growth and sectoring, factors affecting 174–178
  isolates—
    and cultures, color characteristics 170, 171, 184
    morphology 173–174
    relation to russeting of potatoes 181–182
    source and general characteristics 170–172
  pathogenicity, greenhouse tests 178–182, 185
  strains—
    color changes, investigations 170, 171, 184
    studies 169–186
Afanasiev, Michel: A Study of Dormancy and Germination of Seeds of *Cercis canadensis* 405–420
Alfalfa, boron accumulation, factors affecting 248–251, 260, 265, 269, 271
Allium—
  cepa × *A. fistulosum*, smut resistance study 1–7
  fistulosum—
    × *A. cepa*, smut resistance, study 1–7
    smut resistance 1, 2
  spp., hybrid, smut resistance. J. C. Walker, H. A. Jones, and A. E. Clarke 1–8
Allotropa burrelli—
  adult, description 159–160
  egg and parasite body, developmental stages 160–164
  embryonic development, description 159–165
  gregarious parasite of *Pseudococcus comstocki*, biology. D. W. Clancy 159–167
  larva, description 164–165
  life cycle 166
  polyembryony, studies 164
  pupa, description 165
  sex ratio and number of parasites per host 165–166
Andrew, R. H., Brink, R. A., and Neal, N. P.: Some Effects of the Waxy and Sugary Genes on Endosperm Development in Maize 355–371
Animals, farm—
  progeny testing, plan 463–475
  selection for important traits, effectiveness 463–472
  traits—
    measurement only after slaughter 468–470
    of both sexes, measurement before breeding age 463–468
    repeatable, expression only in female 470–472
Apple—
  seedlings, inoculation with *Xylaria mali*, results 449–453
  tree defoliation, effects on susceptibility to *Xylaria mali* infection 454–456
  trees—
    black root rot, host-parasite relations. J. S. Cooley 449–458
    inoculation with black root rot pathogene, studies 450–457
    susceptibility to *Xylaria mali* infection—
      effect of defoliation 454–456
      effect of seasonal maltreatments 453–454

Page
Artichoke, boron accumulation, factors affecting 254–255, 263, 264
Asparagus, boron accumulation, factors affecting 254–255, 263
Aster yellows virus, reaction of *Lactuca* spp. to under field conditions. Ross C. Thompson 110–125

Bacterial ring rot—
  inoculation of tomatoes, method 310–311
  of tomato and eggplant, studies 309–325
  organism—
    on solanaceous hosts 321–323
    See also *Corynebacterium sepedonicum.*
  symptoms in—
    eggplant 321
    tomato 311–312
Bacterium, ring rot, relation to tomato and eggplant. R. H. Larson 309–325
Bakke, A. L., Gaessler, W. G., Pultz, L. M., and Salmon, S. C.: Relation of Cultivation to Depletion of Root Reserves in European Bindweed at Different Soil Horizons 137–147
Barley, boron accumulation, factors affecting 245, 257, 264
Bean—
  kidney, boron accumulation, factors affecting 244–245, 256, 264, 271
  lima, boron accumulation, factors affecting 244–245, 258, 264
  Red Mexican, reaction to curly top virus 153–156
Beet—
  boron accumulation, factors affecting 250–253, 261, 264, 265, 269, 273
  leafhopper—
    development—
      differential effects of temperature on. F. H. Harries 127–136
      effect of temperature, investigations 128–134
    egg development, effect of temperature 128–130
    nymphal development, effect of temperature 128–134
  mosaic, anatomical and cytological studies. Katherine Esau 95–117
  See also Sugar beet.
Beta vulgaris. *See* Beet; Sugar beet.
Bindweed, European—
  carbohydrate content, at various depths in soil, experiments 137–147
  cultivation at different soil horizons, relation to depletion of root reserves. A. L. Bakke, W. G. Gaessler, L. M. Pultz, and S. C. Salmon 137–147
  dry-matter content at various depths in soil, experiments 137–146
  root reserves, depletion at different soil horizons, relation to cultivation. A. L. Bakke, W. G. Gaessler, L. M. Pultz, and S. C. Salmon 137–147
  roots—
    chemical analysis 139
    collection, methods 137–138
    death at various levels 137, 144, 146
    nitrogen content, at various depths in soil, experiments 137–147
    sugar content at different depths in soil, experiments 137–146
Black root rot—
  of apple trees, host-parasite relations. J. S. Cooley 449–458
  See also under host and specific name.
Blackberry, boron accumulation, factors affecting 241, 242–243, 254, 265
Blueberry, highbush—
  growth, effect of soil treatments. T. A. Merrill 9–20
  soil requirements, experiments 11–20

Page

- Bluegrass, Kentucky, boron accumulation, factors affecting 246-247, 258, 269, 271
- Boron—
  - accumulation—
    - in plants—
      - Frank M. Eaton 237-277
      - discussion 267-269
    - *See also* under specific plant.
  - deficiency in plants—
    - Frank M. Eaton 237-277
    - discussion 264-266
    - relation to boron movement 274-275
  - distribution in different parts of plants, discussion 269
  - in plants—
    - effect on growth, with other symptoms, under climatic conditions 242-263
    - study material and methods 237-241
  - movement in plants—
    - examination 269-270
    - relation to deficiency 274-275
  - solubility in leaf tissues 270-274
  - toxicity in plants—
    - Frank M. Eaton 237-277
    - discussion 266-267
- Botanical analysis, point-quadrat method, use on mixed pasture vegetation, studies. William B. Drew 289-297
- BRINK, R. A., ANDREW, R. H., and NEAL, N. P.: Some Effects of the Waxy and Sugary Genes on Endosperm Development in Maize 355-371
- Broadleaf—
  - nurseries—
    - damping-off in Great Plains region. Ernest Wright 77-94
    - in Great Plains region, location, soil, and climate 77-79
  - seedlings—
    - cultural practices in Great Plains region 79-80
    - damping-off—
      - description 80-81
      - hazard in nursery soils 82-83
      - studies in Great Plains region 77-93
  - species—
    - infection with damping-off fungi in sterilized and unsterilized soil, pathogenicity tests 86-92
    - resistant to damping-off, list 82
    - susceptible to damping-off, list 81

- Cabbage, boron accumulation, factors affecting 246-247, 259
- Calendula, boron accumulation, factors affecting 248-249, 259, 271
- California-poppy, boron accumulation, factors affecting 250-251, 261
- *Callistephus chinensis.* *See* Aster.
- Carbohydrate content of—
  - roots of European bindweed at various depths in soil, experiments 137-147
  - sugary and waxy corn kernels investigations 358-361
- CARROLL, W. E., KRIDER, J. L., and FAIRBANKS, B. W.: Value of Soybean Meal Prepared from Damaged (Bin-Burned) Soybeans as a Feed for Growing Swine 383-387
- Carrot, boron accumulation, factors affecting 246-247, 258, 269, 273
- Catalase activity in redbud seed during afterripening 417-419
- Cattle, infestation by liver fluke 401
- Celery, boron accumulation, factors affecting 248-249, 260, 264
- *Cercis canadensis*—
  - seeds, dormancy and germination, study. Michel Afanasiev 405-420
  - *See also* Redbud.
- Cherry, boron accumulation, factors affecting 242-243, 254-255, 265, 268
- Chromosome—
  - behavior in—
    - crossbreeding of strawberries 435-447
    - *Panicum virgatum*, studies 327-351
  - doubling, in maize, studies 47-74
- Chufa, composition, analysis 39, 43, 44, 45

Page

- CLANCY, D. W.: Biology of *Allotropa burrelli*, a Gregarious Parasite of *Pseudococcus comstocki* 159-167
- CLARKE, A. E., JONES, H. A., and WALKER, J. C.: Smut Resistance in an *Allium* Species Hybrid 1-8
- Climate, effect on eggs of *Haemonchus contortus* 285-286
- Color of *Actinomyces scabies* strains, investigations 170, 171, 184
- Connecticut, *Scolytus multistriatus* dispersion, study 301, 304-306
- *Convolvulus arvensis.* *See* Bindweed, European.
- Cooking, effect on color of potato, varietal differences 21-31
- COOLEY, J. S.: Some Host-Parasite Relations in the Black Root Rot of Apple Trees 449-458
- Corn—
  - boron accumulation, factors affecting 246-247, 259
  - canning qualities, effect of sugary and waxy genes, experiments 368-369
  - endosperm development, effects of genetic factors 356-370
  - kernels—
    - carbohydrate content, effect of sugary and waxy genes, investigations 358-361, 369
    - chemical composition at 12 stages of maturity 359
    - moisture content, effect of sugary and waxy genes, investigations 361-362, 369-370
    - sugar content, effect of sugary and waxy genes, experiments 358-360, 369
    - sugary and waxy, histological studies 367-368
    - tenderness, effect of waxy and sugary genes 364-367, 369
    - weight, effect of sugary and waxy genes, investigations 363-364, 369
  - sugary and waxy, canning trials 368-369
  - *See also* Maize.
- *Corynebacterium sepedonicum*—
  - cause of bacterial ring of tomato and eggplant 309, 324-325
  - reaction of tomato varieties to 320
  - *See also* Bacterial ring rot organism.
- Cotton, boron accumulation, factors affecting 254-255, 263, 264
- Cowpea, boron accumulation, factors affecting 244-245, 256, 264, 271
- Curly top—
  - immunity, acquired, in tobacco and tomato. James M. Wallace 187-214
  - plant recovery from, explanatory hypotheses, discussion 210-211
  - virus strains—
    - role in acquired immunity 209-210
    - stability 156
  - *See also* under host.

- Damping-off—
  - fungi—
    - isolation technique 83-84
    - of broadleaf seedlings—
      - hosts and localities 84-86
      - pathogenicity tests through soil inoculation 86-92
  - in broadleaf nurseries of Great Plains region. Ernest Wright 77-94
- Defoliation, apple trees, effect on susceptibility to *Xylaria mali* 454-456
- Desertwillow, susceptibility to damping-off, studies 81, 82, 84, 86, 88
- DICKERSON, G. E., and HAZEL, L. N.: Effectiveness of Selection in Progeny Performance as a Supplement to Earlier Culling in Livestock 459-476
- DINABURG, A. G.: Development and Survival under Outdoor Conditions of Eggs and Larvae of the Common Ruminant Stomach Worm, *Haemonchus contortus* 421-433
- Dispersion, meaning of term 299
- Dormancy of *Cercis canadensis* seeds, study. Michel Afanasiev 405-420
- DREW, WILLIAM B.: Studies on the Use of the Point-Quadrat Method of Botanical Analysis of Mixed Pasture Vegetation 289-297

Page

- Drought, effects on *Stagnicola bulimoides techella* survival 399–400
- Dry-matter content of roots of European bindweed at various depths in soil, experiments 137–146
- Dutch elm disease, vector, dispersion, study 299–307

- Eaton, Frank M.: Deficiency, Toxicity, and Accumulation of Boron in Plants 237–277
- Eggplant—
  - bacterial ring rot, symptoms 321
  - ring rot bacterium relationship. R. H. Larson 309–325
  - varietal resistance to bacterial ring rot organism, studies 323–324
  - varieties susceptible to bacterial ring rot, list 321
- Einset, John, Randolph, L. F., and Abbe, Ernst C.: Comparison of Shoot Apex and Leaf Development and Structure in Diploid and Tetraploid Maize 47–76
- Elm—
  - American, susceptibility to damping-off, studies 81–82, 84, 86, 87, 88
  - bark beetle, smaller European—
    - density regression on distance from center of dispersion. F. M. Wadley and D. O. Wolfenbarger 299–308
    - dispersion center, distance from, regression of density. F. M. Wadley and D. O. Wolfenbarger 299–308
    - dispersion, study 299–307
    - vector of Dutch elm disease 299
  - boron accumulation, factors affecting 242–243, 254, 265
  - damage by *Scolytus multistriatus*, studies in New Jersey and Connecticut 300–306
  - Siberian, susceptibility to damping-off, studies 81–82, 83, 84, 86, 87, 88, 91
- Embryonation of *Haemonchus contortus* eggs, and survival, factors influencing. Doys A. Shorb 279–287
- Energy, free, soil-moisture relations 232–234
- Environment, effect upon larvae of *Haemonchus contortus* 426–430, 431–432
- Esau, Katherine: Anatomical and Cytological Studies on Beet Mosaic 95–117
- *Eutettix tenellus*. *See* Beet leafhopper.

- Fairbanks, B. W., Krider, J. L., and Carroll, W. E.: Value of Soybean Meal Prepared from Damaged (Bin-Burned) Soybeans as a Feed for Growing Swine 383–387
- Farm animals. *See* Animals, farm; Livestock.
- *Fasciola hepatica*. *See* Liver fluke.
- Feces—
  - ruminants, dissemination of *Haemonchus contortus* eggs and larvae 423–425
  - sheep, moisture content, relation to viability of *Haemonchus contortus* eggs 280–286
- Feed for growing swine, value of soybean meal prepared from bin-burned soybeans. J. L. Krider, B. W. Fairbanks, and W. E. Carroll 383–387
- Feedstuffs—
  - American wild, chemical composition. Thomas R. King and Harold E. McClure 33–46
  - wild, composition, investigations 33–46
- Fermentation of tobacco, cigar-leaf, influence of addition of yeast. M. F. Gribbins, D. E. Haley, and J. J. Reid 373–381
- Fertilizer requirements of highbush blueberry plants, experiments 11–20
- Fig, boron accumulation, factors affecting 242–243, 255–256
- Fluke, liver. *See* Liver, fluke.
- *Fragaria*—
  - *ananassa*—
    - × *F. ovalis*, chromosome behavior 437–446
    - crosses, meiotic studies. LeRoy Powers 435–448
  - breeding—
    - allosyndesis 435, 437, 438, 442–443, 445

Fragaria—Continued. Page

  - breeding—Continued.
    - asynapsis 435, 436, 437, 438, 441, 446
    - autosyndesis 435, 437, 438, 442–443, 445
  - *ovalis* crosses, meiotic studies. LeRoy Powers 435–448
  - winter injury, prevention, experiments 435, 445
  - *See also* Strawberry.
- Fruits, wild, feed composition, analysis 38–39, 42–43, 45
- Fungi, damping-off. *See* Damping-off fungi.

- Gaessler, W. G., Bakke, A. L., Pultz, L. M., and Salmon, S. C.: Relation of Cultivation to Depletion of Root Reserves in European Bindweed at Different Soil Horizons 137–147
- Genes—
  - sugary, effects on endosperm development in maize. R. H. Andrew, R. A. Brink, and N. P. Neal 355–371
  - waxy, effects on endosperm development in maize. R. H. Andrew, R. A. Brink, and N. P. Neal 355–371
- Germination, *Cercis canadensis* seeds, study. Michel Afanasiev 405–420
- Giddings, N. J.: Additional Strains of the Sugar-Beet Curly Top Virus 149–157
- Gigas—
  - definition of term 47, 66–67
  - state, ideal, definition 66–67
- Grape, boron accumulation, factors affecting 242–243, 256, 265
- Grass—
  - lespedeza mixture, analysis—
    - by point-quadrat apparatus, comparison of different methods 293–297
    - comparison of methods 289–292
  - species, homogeneity studies 356
- Grasses—
  - point-quadrat determinations in July and August 295–296
  - wild, seed, composition, analysis 36, 41, 44
- Great Plains region, damping-off in broadleaf nurseries. Ernest Wright 77–94
- Gribbins, M. F., Haley, D. E., and Reid, J. J.: The Fermentation of Cigar-Leaf Tobacco as Influenced by the Addition of Yeast 373–381

- *Haemonchus contortus*—
  - common ruminant stomach worm, eggs and larvae, development and survival under outdoor conditions. A. G. Dinaburg 421–433
  - eggs—
    - development and hatching, effects of weather 431–432
    - drying, effects 284–285
    - embryonation and survival, factors influencing. Doys A. Shorb 279–287
    - hatching in laboratory cultures after outdoor exposure 426–430
    - oxygen requirements, investigations 280–282
    - study materials and methods 279–280
    - survival, effect of high and low temperatures 282–284
    - viability, determination by salt flotations 425
  - importance, occurrence, and development 279
  - larvae, development 426–430, 431–432
  - stomach worm, eggs, embryonation and survival, factors influencing. Doys A. Shorb 279–287
- Haley, D. E., Gribbins, M. F., and Reid, J. J.: The Fermentation of Cigar-Leaf Tobacco as Influenced by the Addition of Yeast 373–381
- Harries, F. H.: Differential Effects of Temperature on the Development of the Beet Leafhopper 127–136
- Hazel, L. N., and Dickerson, G. E.: Effectiveness of Selection on Progeny Performance as a Supplement to Earlier Culling in Livestock 459–476

Page

- Hydrogen-ion concentration, effect on *Actinomyces scabies*, experiments 175–178

- Immunity—
  - acquired—
    - from curly top—
      - in tobacco and tomato. James M. Wallace 187–214
      - transfer from tobacco to tomato, studies 190–196, 202–205, 207–208
    - in plants, discussion 205–211
  - from curly top, causation, discussion 206–211
- Insect density, regression on distance from center of dispersion as shown by study of smaller European bark beetle. F. M. Wadley and D. O. Wolfenbarger 299–308
- Irrigation, moisture retention in relation to soil-moisture tension. L. A. Richards and L. R. Weaver 215–235

- Jerusalem-artichoke, boron accumulation, factors affecting 244–245, 256–257, 274
- Jones, H. A., Walker, J. C., and Clarke, A. E.: Smut Resistance in an *Allium* Species Hybrid 1–8

- King, Thomas R., and McClure, Harold E.: Chemical Composition of Some American Wild Feedstuffs 33–46
- Krider, J. L., Fairbanks, B. W., and Carroll, W. E.: Value of Soybean Meal Prepared from Damaged (Bin-Burned) Soybeans as a Feed for Growing Swine 383–387

- *Lactuca*—
  - spp., reaction to aster yellows virus under field conditions. Ross C. Thompson 119–125
  - *See also* Lettuce.
- Larkspur, boron accumulation, factors affecting 244–245, 257
- Larson, R. H.: The Ring Rot Bacterium in Relation to Tomato and Eggplant 309–325
- Leafhopper—
  - beet, development, differential effects of temperature on. F. H. Harries 127–136
  - transmission of aster yellows 119
- Legume, wild, seeds, composition, analysis 35, 40, 44
- Lemon, boron accumulation, factors affecting 242–243, 254, 269
- Lespedeza—
  - panicum mixture, analysis—
    - by point-quadrat apparatus, comparison of different methods 293–297
    - methods, comparison 289–292
  - point-quadrat determinations in July and August 294–295
- Lettuce—
  - boron accumulation, factors affecting 250–251, 260, 271
  - varieties—
    - cultivated, reaction to aster yellows under field conditions 120, 121–122
    - reaction to aster yellows under field conditions, experiments 120–124
    - wild, reaction to aster yellows under field conditions 120–121, 122–124
  - See also *Lactuca*.
- Lime, application to blueberry plants, experiments 12–14
- Liver fluke—
  - importance in Gulf coast region 389
  - infestation in cattle and sheep 401
  - intermediate host, lymnaeid snail, *Stagnicola bulimoides techella*, bionomics in southern Texas. O. Wilford Olsen 389–403
- Livestock—
  - culling—
    - earlier, supplement in effectiveness of selection on progeny performance. G. E. Dickerson and L. N. Hazel 459–476
    - first, result in genetic superiority 461–462
    - second, addition to genetic superiority 462–463
  - improvement, annual, from selection in closed populations 459–461

Page

- Livestock—Continued.
  - progeny performance, effectiveness of selection as supplement to earlier culling. G. E. Dickerson and L. N. Hazel 459–476
  - selection—
    - effectiveness on progeny performance as supplement to earlier culling. G. E. Dickerson and L. N. Hazel 459–476
    - for important traits, effectiveness 463–472
- Locust, black, susceptibility to damping-off, studies 81, 82, 84, 86, 88, 89–90
- Lupine, boron accumulation, factors affecting 242–243, 256
- Lymnaeid snail. *See* Snail, lymnaeid.

- *Macrosteles divisus*, transmission of aster yellows virus 119
- Maize—
  - autotetraploids, morphological analyses 48
  - chromosome doubling, studies 47–74
  - diploid—
    - and tetraploid, shoot apices—
      - and leaves, comparative relations 66–68
      - formation and size relations during plastochron 10, data 49–52
    - leaf blade, mature tenth—
      - histology 59–64
      - morphology 56–59
      - total number of cells, estimation 64–66, 73–74
    - leaf growth, rate of increase, relation to tetraploid 52–56
    - shoot apex and leaf development and structure, comparison with tetraploid maize. L. F. Randolph, Ernst C. Abbe, and John Einset 47–76
    - tenth leaf blade, development, comparison with tetraploid 52–56
  - endosperm development, effects of waxy and sugary genes. R. H. Andrew, R. A. Brink, and N. P. Neal 355–371
  - genes, waxy and sugary, effect on endosperm development. R. H. Andrew, R. A. Brink, and N. P. Neal 355–371
  - tetraploid—
    - and diploid, shoot apices and leaves, comparative relations 66–68
    - leaf blade—
      - chimera, comparison with diploid 56–57, 64, 66
      - mature tenth, histology 59–64
      - mature tenth, morphology 56–59
      - mature tenth, total number of cells, estimations 64–66, 73–74
    - leaf growth, rate of increase, comparison with diploid 52–56
    - shoot apex and leaf development and structure, comparison with diploid maize. L. F. Randolph, Ernst C. Abbe, and John Einset 47–76
    - tenth leaf blade, development, comparison with diploid maize 52–56
  - tetraploidy in relation to genotypic constitution and gigas state 47, 68–69, 70, 74
  - *See also* Corn.
- Mast, composition, analysis 37, 41–42, 44–45
- McClure, Harold E., and King, Thomas R.: Chemical Composition of Some American Wild Feedstuffs 33–46
- McFarlane, John S., Rieman, G. H., and Tottingham, W. E.: Potato Varieties in Relation to Blackening after Cooking 21–31
- Mealybug, Comstock. See *Pseudococcus comstocki*.
- Meiosis in crosses between *Fragaria ovalis* and × *F. ananassa*. LeRoy Powers 435–448
- Merrill, T. A.: Effects of Soil Treatments on the Growth of the Highbush Blueberry 9–20
- Micro-organisms, development, effect on tobacco flavor, experiments 374–381
- Milo, boron accumulation, factors affecting 246–249, 259, 264

- Moisture—
  - content of sugary and waxy corn kernels, investigations 361–362, 369–370
  - retention by irrigated soils, relation to soil-moisture tension. L. A. Richards and L. R. Weaver 215–235

Page
Mosaic, beet, anatomical and cytological studies. Katherine Esau 95–117
Muck, growing highbush blueberries in, experiments 15–20
Muriate of potash, effect upon highbush blueberry plants, experiments 15
Muskmelon, boron accumulation, factors affecting 252–253, 261, 264, 272, 273
Mustard, boron accumulation, factors affecting 248–249, 260

NEAL, N. P., ANDREW, R. H., and BRINK, R. A.: Some Effects of the Waxy and Sugary Genes on Endosperm Development in Maize 355–371
New Jersey, *Scolytus multistriatus* dispersion, study 301–304, 306
NIELSEN, ETLAR L.: Analysis of Variation in *Panicum virgatum* 327–353
Nurseries, broadleaf. *See* Broadleaf nurseries.
Nutritive value of wild feedstuffs, analyses 33–46

Oats, boron accumulation, factors affecting 248–249, 260
OLSEN, O. WILFORD: Bionomics of the Lymnaeid Snail, *Stagnicola bulimoides techella*, the Intermediate Host of the Liver Fluke in Southern Texas 389–403
Onion—
  boron accumulation, factors affecting 244–247, 258, 265, 269, 273
  hybrid, smut resistance, experiments 1–7
Oxalis, boron accumulation, factors affecting 252–253, 262–263
Oxygen—
  effect on germination of afterripened redbud seeds 414–415
  requirements of *Haemonchus contortus*, investigations 280–282

Panicum-lespedeza mixture, analysis—
  by point-quadrat apparatus, comparison of different methods 293–297
  comparison of methods 289–292
Panicum virgatum—
  characters, regression, covariance studies 342–344
  chromosome—
    distribution 344–347
    fixation and stains 328
    number—
      and morphology 329–332
      and sources 327–328
  gross morphology, relation to chromosome number 332–341
  isolates, statistical treatment 329
  morphological measurements 328–329
  plant height and panicle length, covariance with other characters 342–344
  polyploidy 329–332
  variation, analysis. Etlar L. Nielsen 327–353
Pansy, boron accumulation, factors affecting 242–243, 256
Parsley, boron accumulation, factors affecting 248–249, 260
Pasture vegetation—
  analysis, comparison of methods 289–292
  mixed, botanical analysis by use of point-quadrat method, studies. William B. Drew 289–297
Pea, boron accumulation, factors affecting 244–245, 258, 272–273
Peach, boron accumulation, factors affecting 242–243, 255, 268
Persimmon, boron accumulation, factors affecting 242–243, 255, 265
Physiologic specialization in common scab fungus, *Actinomyces scabies*. L. A. Schaal 169–186
Pigs—
  feed value of soybean meal prepared from bin-burned soybeans, experiments 383–387
  See also Swine.

Page
Plant—
  hosts, sugar-beet curly top virus, classified list 150–151
  leaf tissue, boron accumulation, solubility 270–274
Plants—
  boron—
    accumulation—
      and tolerance 269
      characteristics, diversity in different varieties, experiments 238–276
      climatic effects 269
      movement and deficiency, correlations 274–275
    deficiency—
      analysis 264–266
      study material and methods 237–241
      toxicity, and accumulation. Frank M. Eaton 237–277
    semi-tolerant, list 244–251
    sensitive, list 242–245
    tolerant, list 250–255
    traces and movement 269–270
  immunity from curly top, causation, discussion 206–211
  recovery from curly top, explanatory hypotheses, discussion 210–211
  resistant to sugar-beet curly top, list 151
  wilting—
    point, osmotic concentration of soil solution 228–229
    range, soil moisture movement 231–232
Plastochron, meaning of term 49
Point-quadrat method of botanical analysis of mixed pasture vegetation, studies on use. William B. Drew 289–297
Polyploidy in *Panicum virgatum* 329–332
Potato—
  boron accumulation, factors affecting 246–247, 259, 264
  scab fungus, variation and physiologic specialization, experiments 169–186
  varieties—
    blackening after cooking. G. H. Rieman, W. E. Tottingham, and John S. McFarlane 21–31
    reaction to scab in different localities 182, 185
    susceptibility to *Actinomyces scabies*, comparison 171
Potatoes—
  cooked—
    blackening—
      environmental influences 29–30
      genetic influences 27–29
      of hybrid populations 24–25
      relation to location 25
      relation to season 25–26
      relation to varieties and strains 23–24
  russeting, investigations 181–182
POWERS, LEROY: Meiotic Studies of Crosses between *Fragaria ovalis* and × *F. ananassa* 435–448
Pressure-membrane apparatus, use in soil-moisture tests 215–234
Progeny testing, livestock, plan 463–475
Pseudococcus comstocki—
  gregarious parasite of, *Allotropa comstocki*, biology. D. W. Clancy 159–167
  pest of apple trees 159
PULTZ, L. M., BAKKE, A. L., GAESSLER, W. G., and SALMON, S. C.: Relation of Cultivation to Depletion of Root Reserves in European Bindweed at Different Soil Horizons 137–147
Pythium ultimum, cause of damping-off of broadleaf seedlings 84, 86, 87–92

Radish, boron accumulation, factors affecting 248–249, 259–260, 264, 265, 269, 273, 274–275
Rams, infection with *Haemonchus contortus*, experiments 423–433
RANDOLPH, L. F., ABBE, ERNST C., and EINSET, JOHN: Comparison of Shoot Apex and Leaf Development and Structure in Diploid and Tetraploid Maize 47–76

Page
Redbud—
  seed—
    acid treatment, effects on germination ... 407–411
    afterripening and germination—
      effects of various treatments on, data. 407–412
      microchemical tests ... 412–413
    catalase activity during afterripening ... 417–419
    chemical treatments, effects on germination ... 407–412
    dormancy and germination studies ... 405–419
    enzymes, microchemical tests ... 412–413
    germination—
      factors affecting ... 413–416
      forcing by seed treatments ... 407–412
      microchemical changes during ... 413–414
    stratification period, length in relation to germination after seed treatments ... 409–412
    sulfuric acid treatment, effects on germination ... 407–411
  See also *Cercis canadensis*.
Redpepper, boron accumulation, factors affecting ... 246–247, 258
Reid, J. J., Gribbins, M. F., and Haley, D. E.: The Fermentation of Cigar-Leaf Tobacco as Influenced by the Addition of Yeast ... 373–381
Rhizoctonia solani, cause of damping-off of broadleaf seedlings ... 84–92
Richards, L. A., and Weaver, L. R.: Moisture Retention by Some Irrigated Soils as Related to Soil-Moisture Tension ... 215–235
Rieman, G. H., Tottingham, W. E., and McFarlane, John S.: Potato Varieties in Relation to Blackening after Cooking ... 21–31
Ring rot—
  bacteria—
    movement and distribution in tomato tissue ... 312–317
    transmission—
      by tomato seed, investigations ... 317–318
      through inoculated soil ... 318–320
  bacterium, relation to tomato and eggplant. R. H. Larson ... 309–325
Ruga verrucosans. *See* Sugar-beet curly top virus.
Ruminants, parasitization by *Haemonchus contortus* ... 279

Saccharomyces cerevisiae, use in fermentation of cigar-leaf tobacco, experiments ... 374–381
Salmon, S. C., Bakke, A. L., Gaessler, W. G., and Pultz, L. M.: Relation of Cultivation to Depletion of Root Reserves in European Bindweed at Different Soil Horizons ... 137–147
Sand, Saugatuck, growing highbush blueberries in, experiments ... 11–12, 15–20
Scab, common, fungus—
  Actinomyces scabies, variation and physiological specialization. L. A. Schaal ... 169–186
  See also *Actinomyces scabies*.
Schaal, L. A.: Variation and Physiological Specialization in the Common Scab Fungus, *Actinomyces scabies* ... 169–186
Scolytus multistriatus. *See* Elm bark beetle, smaller, European.
Seedlings, broadleaf. *See* Broadleaf seedlings.
Seeds—
  of *Cercis canadensis*, dormancy and germination, study. Michel Afanasiev ... 405–420
  wild, nutritive value, analyses ... 33–46
Selection, effectiveness on progeny performance as supplement to earlier culling in livestock. G. E. Dickerson and L. N. Hazel ... 459–476
Sheep—
  infection by—
    Haemonchus contortus, climatic factors ... 282–286
    liver fluke, factors involving intermediate host ... 401
  parasite—
    Haemonchus contortus, development and survival, experiments ... 421–433
    liver fluke, embryonation and survival, experiments ... 279–286
  progeny testing plan ... 463–475

Page
Shorb, Doys A.: Factors Influencing Embryonation and Survival of Eggs of the Stomach Worm, *Haemonchus contortus* ... 279–287
Smut resistance in an *Allium* spp. hybrid. J. C. Walker, H. A. Jones, and A. E. Clarke ... 1–8
Snail—
  habitats, types and characteristics ... 393–395
  lymnaeid—
    intermediate host of liver fluke in southern Texas, bionomics. O. Wilford Olsen ... 389–403
    See also *Stagnicola bulimoides techella*.
Soil—
  acidity, requirements of highbush blueberry plants, experiments ... 9–20
  conditions, effects on *Stagnicola bulimoides techella* survival ... 399–400
  effect upon strains of *Actinomyces scabies* ... 175–178, 185
  horizons, European bindweed cultivation, relation to depletion of root reserves. A. L. Bakke, W. G. Gaessler, L. M. Pultz, and S. C. Salmon ... 137–147
  inoculation with damping-off fungi in tests of broadleaf seedlings ... 86–92
  moisture—
    energy relations, experimental results ... 232–234
    relations in 1-atmosphere range, determination ... 225–228
    retention curve, use ... 222–225
    tension—
      and moisture equivalent relations, tests ... 221
      in wilting range, determination ... 228–230
      of irrigated soils, relation to moisture retention. L. A. Richards and L. R. Weaver ... 215–235
    testing—
      apparatus, description and operation. 215–218
      by use of pressure-membrane apparatus, experiments ... 215–234
  muck, growing highbush blueberries in, experiments ... 15–20
  solution, osmotic concentration at wilting point ... 228–229
  transmission of bacterial rot organism ... 318–320
  treatments, effects on growth of highbush blueberry. T. A. Merrill ... 9–20
Soils—
  irrigated, moisture retention, relation to soil-moisture tension. L. A. Richards and L. R. Weaver ... 215–235
  moisture—
    extraction, fifteen-atmosphere percentage and wilting range, determinations ... 218–221
    movement in wilting range, determinations by use of pressure-membrane apparatus ... 231–232
    retention—
      after moisture-extracting treatments, determination ... 218–221
      effects of temperature ... 222
      plotting curves ... 222–225
  nursery, damping-off hazard in Great Plains ... 82–83
  pore-size, relation to moisture retention, studies ... 222–225
Solanaceae, hosts of ring rot organism, list ... 321–323
Solanum—
  melongena. *See* Eggplant.
  tuberosum. *See* Potato; Potatoes.
Soybean meal prepared from damaged (bin-burned) soybeans, value as feed for growing swine. J. L. Krider, B. W. Fairbanks, and W. E. Carroll ... 383–387
Soybeans, damaged (bin-burned), meal prepared from, value as feed for growing swine. J. L. Krider, B. W. Fairbanks, and W. E. Carroll ... 383–387
Stagnicola bulimoides techella—
  desiccation and unfavorable temperatures, effect on survival ... 399–400
  dissemination methods ... 400–401
  egg-production data ... 398
  in Gulf coast prairie of Texas, habitat types ... 393–395

- *Stagnicola bulimoides techella*—Continued. Page
  - in southern Texas, bionomics 391–402
  - intermediate host of liver fluke in southern Texas, bionomics. O. Wilford Olsen 389–403
  - life—
    - cycle, studies 391–392
    - history 395–398
  - natural infection with *Fasciola hepatica*, data 401
  - *See also* Snail, lymnaeid.
- Stomach worm. See *Haemonchus contortus*.
- Strawberry—
  - boron accumulation, factors affecting 242–243, 256
  - breeding by hybridization method, problems 441–445
  - crosses, meiotic studies 435–447
  - Fairfax. See *Fragaria ananassa*.
  - hybrids, chromosome pairing 435–436, 442–443, 445–446
  - Rocky Mountain, crossing studies 435–447
  - winter injury, prevention by propagation, experiments 435, 445
  - See also *Fragaria*.
- Sugar content of—
  - roots of European bindweed at various depths in soil, experiments 137–146
  - sugary and waxy corn kernels, experiments 358–360, 369
- Sugar beet—
  - boron accumulation, factors affecting 252–253, 261–262, 265, 271
  - chloroplasts, mosaic-infected, characteristics 105–110
  - curly top—
    - plants resistant to, list 151
    - severity grades, original and revised, descriptions, comparisons 153
    - strains 5 and 6, differentiation 153–156
    - virus hosts, classified list 150–151
    - virus strains—
      - N. J. Giddings 149–157
      - differentiation methods 151–153
      - sources 149
  - leaves—
    - healthy, phloem, structure 97–98
    - mesophyll, effect of mosaic 100–101, 111–112
    - mosaic-diseased phloem, structure 98–100
    - mottling, relation to mosaic 100–101, 112
    - plastids, effect of mosaic 101–110, 112–114
  - mosaic—
    - infected, yellow and green areas, comparison 110–112
    - studies, materials, and methods 96
    - symptoms, external 96–97
  - *See also* Beet.
- Sweet pea, boron accumulation, factors affecting 252–253, 261, 264–265
- Sweetclover, boron accumulation, factors affecting 252–253, 261, 269
- Sweetpotato, boron accumulation, factors affecting 244–245, 258, 264, 272–273
- Swine—
  - growing, feed, value of soybean meal from bin-burned soybeans. J. L. Krider, B. W. Fairbanks, and W. E. Carroll 383–387
  - progeny testing, plan 464–475
- Switchgrass. See *Panicum virgatum*.

- Temperature, effects, differential, on development of beet leafhopper. F. H. Harries 127–136
- Texas—
  - Gulf coast prairie, characteristics 392–393
  - southern, lymnaeid snail, *Stagnicola bulimoides techella*, intermediate host of liver fluke, bionomics. O. Wilford Olsen 389–403
- THOMPSON, ROSS C.: Reaction of *Lactuca* Species to the Aster Yellows Virus under Field Conditions 119–125
- Tobacco—
  - boron accumulation, factors affecting 250–251, 260, 272
  - cigar-leaf—
    - fermentation, influence of addition of yeast. M. F. Gribbins, D. E. Haley, and J. J. Reid 373–381
    - fermenting, temperature changes, relation to yeast treatment 376, 379
- Tobacco—Continued. Page
  - cigar leaf—Continued.
    - microflora—
      - after fermentation with yeast 377
      - before fermentation 375, 379, 380
  - curly top, immunity. James M. Wallace 187–214
  - fermentation, experiments 373–381
  - immunity—
    - acquired, from curly top. James M. Wallace 187–214
    - from curly top—
      - discussion 206–209, 211
      - transfer to tomato, studies 190–196, 202–205, 207–208
  - plants—
    - healthy, back grafts from tomato passively immunized against curly top 193–195
    - topping, effect upon curly top activity 208
    - use in immunizing tomato against curly top, methods 190–193
  - Turkish, reaction to curly top virus 153, 156
- Tomato—
  - boron accumulation, factors affecting 250–251, 261, 272
  - clones passively immunized against single known virus strains, reaction to different virus strains 202–205
  - curly top, immunity—
    - James M. Wallace 187–214
    - acquired—
      - from tobacco, studies 190–196, 202–205, 207–208
      - role of virus strains 199–202
  - immunity—
    - acquired, from curly top. James M. Wallace 187–214
    - from curly top, discussion 207–208, 209–210, 211
  - immunization from curly top, passive, by means of tobacco plants, methods 190–193
  - plants—
    - bacterial ring rot symptoms 311–312
    - immunized against curly top—
      - actively, reaction to different virus strains, experiments 199–202
      - actively, study 188–190
      - controlled inoculations with virus, experiments 198–199
      - field tests 196–199
      - natural exposure to virus, experiments 196–198
      - passively, back grafts to healthy tobacco plants 193–195
      - passively, greenhouse reinoculation tests 195–196
      - passively, reaction to different virus strains 202–205
    - inoculation by ring rot bacterium, method 310–311
  - ring rot bacterium relationship. R. H. Larson 309–325
  - seed, transmission of bacterial ring rot organism 317–318
  - tissue, break-down due to ring rot infection, studies 312–317
  - varieties, reaction to *Corynebacterium sepedonicum* 320
- TOTTINGHAM, W. E., RIEMAN, G. H., and MCFARLANE, JOHN S.: Potato Varieties in Relation to Blackening after Cooking 21–31

- *Vaccinium corymbosum*. *See* Blueberry, highbush.
- Vegetation, pasture—
  - analysis, comparison of methods 289–292
  - mixed, botanical analysis by use of point-quadrat method, studies. William B. Drew 289–297
- Vetch, boron accumulation, factors affecting 250–251, 260–261
- Violet, boron accumulation, factors affecting 242–243, 256
- Virus—
  - diseases, immunity, acquired, in plants, discussion 205–211
  - strains of sugar-beet curly top. N. J. Giddings 149–157
- Vitamin B_1, effects on redbud seed germination, experiments 412, 415

Page

WADLEY, F. M., and WOLFENBARGER, D. O.: Regression of Insect Density on Distance from Center of Dispersion as Shown by a Study of the Smaller European Elm Bark Beetle ... 299–308

WALKER, J. C., JONES, H. A., and CLARKE, A. E.: Smut Resistance in an *Allium* Species Hybrid ... 1–8

WALLACE, JAMES M.: Acquired Immunity from Curly Top in Tobacco and Tomato. 187–214

Water retention in soils, energy relations ... 232–234

Weather, effect on development and hatching of *Haemonchus contortus* eggs ... 431–432

WEAVER, L. R., and RICHARDS, L. A.: Moisture Retention by Some Irrigated Soils as Related to Soil-Moisture Tension. 215–235

Wildlife, feedstuffs, chemical composition, analyses ... 33–46

WOLFENBARGER, D. O., and WADLEY, F. M.: Regression of Insect Density on Distance from Center of Dispersion as Shown by a Study of the Smaller European Elm Bark Beetle ... 299–308

Page

WRIGHT, ERNEST: Damping-off in Broadleaf Nurseries of the Great Plains Region. 77–94

Xylaria mali—
- cause of black root rot of apple trees ... 449
- infection of apple trees—
  - effect of seasonal maltreatments ... 450–454
  - host-parasite relation ... 449–458
- inoculation of apple seedlings at monthly intervals, effects ... 450–453
- *See also* Black root rot; and under host.

Yeast, addition to cigar-leaf tobacco, influence on fermentation. M. F. Gribbins, D. E. Haley, and J. J. Reid ... 373–381

Yellows, aster, virus, reaction of *Lactuca* spp. to under field conditions. Ross C. Thompson ... 119–125

Zinnia, boron accumulation, factors affecting ... 244–245, 257

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before ....

Return Date	Return Date